Metabolism of polycyclic aromatic hydrocarbons by the microbiome of the human skin

Inaugural-Dissertation

to obtain the academic degree

Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry and Pharmacy

of Freie Universität Berlin

by

Juliane Sowada

from Magdeburg

2017

Die vorliegende Arbeit wurde von Oktober 2011 bis einschließlich September 2017 unter der Leitung von Prof. Dr. Dr. Andreas Luch am Bundesinstitut für Risikobewertung in Berlin angefertigt.

1. Gutachter: Prof. Dr. Dr. Andreas Luch
2. Gutachter: Prof. Dr. Rupert Mutzel

Tag der Disputation: 24.01.2018

Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel „Metabolism of polycyclic aromatic hydrocarbons by the microbiome of the human skin“ selbstständig und nur unter der Verwendung angegebener Hilfsmittel und Literatur angefertigt habe. Wörtlich oder sinngemäß entnommene Stellen aus der Literatur und aktuellen Publikationen habe ich als solche kenntlich gemacht. Die vorliegende Arbeit wurde in keinem früheren Promotionsverfahren angenommen oder als ungenügend beurteilt.

Berlin, 2017

Acknowledgement - Danksagung

Herrn Prof. Dr. Dr. Andreas Luch danke ich für die Möglichkeit der Anfertigung der Promotion, die sehr gute experimentelle Ausstattung, die mir dafür zur Verfügung stand und das Vertrauen, mir ein sehr interessantes und aufstrebendes mikrobiologisches Thema in einer Zellkultur geprägten Umgebung zu überlassen. Ich bedanke mich bei Ihnen für die Möglichkeit mein Thema frei und kreativ bearbeiten zu dürfen.

Herrn Prof. Dr. Mutzel möchte ich danken für die Übernahme der Funktion als Zweitgutachters und der damit verbundenen zusätzlichen Arbeit.

Ein besonderer Dank für die initiale Planung und die Kreativität des Themas gilt meinem Betreuer Herrn Dr. Tewes Tralau. Ohne ihn wäre dieses Thema nicht möglich gewesen. Vielen herzlichen Dank für die wissenschaftliche Unterstützung beim Erstellen der Arbeit, das kritische Beäugen meiner wissenschaftlichen Arbeit und die konstruktiven Anregungen – manchmal macht's der Blickwinkel. Dankeschön auch für die stete positive Blickrichtung, die immer wieder für neue Energie und Motivation gesorgt hat.

Ich möchte auch Herrn Dr. Christoph Hutzler, Herrn Dr. Ingo Ebner und Karsten Schön aus der Analytik danken, denen die nicht ganz einfache Aufgabe zu Teil wurde mir in allen analytischen Fragen mit Rat und Tat zur Seite zu stehen.

Der FAZIT-Stiftung möchte ich danken für die Übernahme der Finanzierung der Abschlussphase meiner Doktorarbeit.

Ein herzliches Dankeschön auch an die gesamte experimentelle Gruppe aus der 6. Etage, die sich nach der Umstrukturierung nun mehr in den unterschiedlichsten Fachgruppen wiederfinden. Danke für die Akzeptanz von bakteriellen Kulturen in einer Zellkulturumgebung, die Hilfe bei der täglichen Suche nach Laborzubehör und Chemikalien, den guten Ratschlägen beim Experimentieren und ‚Geräte-Bedienen-Lernen' unter QM und natürlich für die amüsanten und interessanten Flurgespräche. Insbesondere Doris Genkinger möchte ich danken für die tolle Einarbeitung in die Zellkultur, die stete Hilfe und die interessanten Gespräche.

Natürlich möchte ich auch allen Probanden und ihrer mannigfaltigen Hautmikrobiota dafür danken, dass sie sich zur Verfügung gestellt haben und mir ein Teil ihres wertvollen Hautmikrobioms zum Experimentieren überlassen haben. Ohne Sie/ Euch und Ihr/ Euer tolles Hautmikrobiom wäre das ganze Projekt praktisch nicht möglich gewesen!

Einen ganz besonderen Dank an meine Eltern, die auch in unwägbaren Zeiten immer einen Weg gefunden haben, mich in dem was ich tue zu unterstützen und zu stärken auch wenn ich trotz Doktorarbeit nicht in der Lage bin Krankheiten zu diagnostizieren und zu behandeln.

Abschließend ein besonders großes Dankeschön an meine kleine Familie. Vielen lieben Dank Svennie, für die Motivation und Unterstützung. Danke, dass Du wie selbstverständlich alle Aufgaben übernommen hast an den unzähligen Laborwochenenden und –abenden. Danke, dass Du mir geholfen hast, als gar nichts mehr ging und in jeder noch so schwierigen Situation an meiner Seite stehst. Danke Svennie und Erik, dass es Euch Beide gibt!

Content

Abbreviations

%	Percent
AHR	Arylhydrocarbonreceptor
AMP	Antimicrobial peptides
ARNT	Aryl hydrocarbon receptor nuclear translocator protein
B[*a*]A	Benzo[*a*]anthracene
B[*a*]A-diole	Benzo[*a*]anthracenediole
B[*a*]A-1-ol	1-Hydroxybenzo[*a*]anthracene
B[*a*]A-11-ol	11-Hydroxybenzo[*a*]anthracene
B[*a*]P	Benzo[*a*]pyrene
B[*a*]P-1,6-dione	Benzo[*a*]pyrene-1,6-dione
B[*a*]P-6,12-dione	Benzo[*a*]pyrene-6,12-dione
B[*a*]P-7,8-dione	Benzo[*a*]pyrene-7,8-dione
B[*a*]P-1-ol	1-Hydroxybenzo[*a*]pyrene
B[*a*]P-2-ol	2-Hydroxybenzo[*a*]pyrene
B[*a*]P-3-ol	3-Hydroxybenzo[*a*]pyrene
B[*a*]P-4-ol	4-Hydroxybenzo[*a*]pyrene
B[*a*]P-7-ol	7-Hydroxybenzo[*a*]pyrene
B[*a*]P-10-ol	10-Hydroxybenzo[*a*]pyrene
B[*a*]P-12-ol	12-Hydroxybenzo[*a*]pyrene
bHLH	basic Helix Loop Helix Protein
BPDE	Benzo[*a*]pyrene-7,8-diole-9,10-epoxide
BLAST	Basic local alignment search tool
β-NF	β-Naphthoflavone
°C	Degree celcius
cm^2	Square centimeter

CYP	Cytochrom P450–dependent monooxygenase
DB[*a,l*]P	Dibenzo[*a,l*]pyrene
DNA	Deoxyribonucleic acid
DszaA/NtaA	Dibenzothiophensulfone/ Ntritilacetate monooxygenase
EC_{50}	Half maximal effective concentration
e.g.	for example
EYFP	Enhanced yellow fluorescent protein
Fe	Iron
Fig	Figure
g	Gram
GABA	ɤ Aminobutyric acid
GC-MS	Gas chromatography coupled to mass spectrometry
HaCaT	Human adult low Calcium high Temperature cells
HepG2	Hepatoma G2 cells
HMW	High molecular weight
Hsp90	Heat shock protein 90
LB	Lysogeny broth
LC-MS	Liquid chromatography coupled to mass spectrometry
LMW	Low molecular weight
m^2	Square meter
min	Minute
µM	Micromolar
mRNA	Messenger ribonucleic acid
NAD(P)H	Nicotinamide adenin dinucleotide (phosphate)
NES domain	Nuclear export signal domain
NHEK	Normal human epidermal keratinocytes

NifH	Gene of the nitrogen reductase
NLS domain	Nuclear localisation signal domain
nM	Nanomolar
p23	Prostaglandine E Synthase 3
PAH	Polycyclic aromatic hydrocarbon
PAS	Period circadian protein-aryl hydrocarbon receptor nuclear translocator protein- single-minded protein
Phe-4-CO_2H	Phenanthrene-4-carboxylic acid
RHO	Ring hydroxylating oxygenase
rRNA	Ribosomal ribonucleic acid
SCFA	Short chain fatty acids
SU	Sub unit
US-EPA	United States environmental protection agency
XAP2	Hepatitis B virus X-associated protein 2
XRE	Xenobiotic response element

List of figures

1. Introduction

1.1 The Skin – A Sensory and Protective Organ

With an area of 2 m^2, the skin is the largest human sensory organ. Biologically, the skin is subdivided into three layers (Butnaru and Kanitakis 2002):

- Epidermis
- Dermis
- Hypodermis (subcutis).

The epidermis is the outmost layer of the skin. It is exposed to the outside world, forms a physical and metabolic barrier which, in addition to its function as an enclosing organ, serves primarily to protect against water loss and environmental influences. The more sensitive internal organs are protected against biotic and abiotic influences as well as UV radiation (Nemes and Steinert 1999, Madison 2003). Constantly self-renewing keratinocytes form a squamous epithelium of 60 – 180 µm thickness (Loewenthal 1963). In addition to these squamous cells, dendritic melanocytes are present in the epidermis along with Langerhans and Merkel cells. Melanocytes are responsible for melanin synthesis, i.e. they synthesise the pigments that give the skin its colour, thereby forming a protection against aggressive UV light (Kollias *et al.* 1991). Langerhans cells are inactive dendritic cells which are capable of phagocytising permeating antigens. After making antigen contact, the Langerhans cells differentiate into mature dendritic cells which migrate via the bloodstream to the lymph nodes. Here they trigger immune reactions mainly through interaction with T-lymphocytes (Kaplan *et al.* 2008). On the other hand, Merkel cells function as mechanoreceptors that pass on haptic stimuli (Aldag *et al.* 2016).

The dermis, which is made up of a mesh of various glands (sweat and sebaceous glands), sensory receptors (temperature, pain, touch), blood vessels, and muscle fibres, is located below the epidermis (Loewenthal 1963). It forms a connective tissue of elastin and collagen fibres that produces the mechanical stability of the skin. In addition to providing the epidermis with nutrients, the dermis provides immune cells, such as macrophages, lymphocytes, plasma cells, mast cells, granulocytes and monocytes (Elias 1981).

The subcutis, the inner layer of the skin, consists of loose connective tissue whose characteristic feature is the depositing of fat cells. In addition, many of the blood vessels required for

supplying the skin with nutrients are located in the subcutis. Due to the large number of fat cells, this skin layer serves mainly as an energy reserve, heat protection, and as32 a mechanical buffer for the human organism (Kanitakis 2002).

In addition to its protective function, the skin helps to regulate the body-heat balance and is actively involved in the transport of various substances via the blood vessels in the dermis and subcutis (Ley 2015). Urinary excreted substances such as sodium chloride are segregated via the sweat glands. Moreover, Vitamin D_3 which regulates calcium and phosphate levels is produced in the epidermis under the influence of sunlight (Holick 1995, Lips 2006). Furthermore, lipophilic substances which diffuse via the horny layer or skin adnexa, such as sebaceous and sweat glands, are resorbed through the skin (Karzel and Liedtke 1989). With millions of sensory receptors, the skin is also the primary touch and sensory organ. In this way, the environment can be perceived via pain, touch or thermo receptors and an adequate response given to incoming stimuli (Potter 1966). In addition, the skin is important for triggering immune reactions and protecting against the intrusion of pathogenic microorganisms (Yazdi *et al.* 2016). Various molecules which can directly prevent bacterial growth or have an activating effect on the human immune system are synthesised by the epithelial cells during this process, such as antimicrobial peptides (AMP), proteases, cytokines and chemokines (Christensen and Bruggemann 2014).

Together with the skin, the microbiome is responsible for protection against pathogens and initiation of immune reactions (Findley and Grice 2014, Grice 2014, Kuk *et al.* 2016).

1.2 The Human Microbiome – the human body as a host

1.2.1 Composition of the skin microbiome

Humans are inhabited by millions of microorganisms known as the microbiome. It is made up of various prokaryotes and eukaryotes which have their natural habitat on the diverse surfaces on and in the human body (Grice and Segre 2012, Hannigan and Grice 2013, The human microbiome project consortium 2012). A comparison with the human genome shows that the microbiome has over 150 times more genes than the human genome (Backhed 2012, Grice and Segre 2012). Approx. 40,000 different species exist, spread over 1,800 genera (Arumugam *et al.* 2011, Grice and Segre 2012). The majority of this biomass is located on the skin and in the

gastrointestinal tract, the organs that also provide the largest surface area for colonisation (Costello *et al.* 2009, Grice and Segre 2012). Separate ecosystems are created by differential colonisation of the various habitats that the human body provides as a host (Costello *et al.* 2012, Grice *et al.* 2008).

The skin is subdivided into three large habitats: dry, moist, and sebaceous (Jarvis 1994, Leser and Molbak 2009). In a similar way to the microbial colonisation of other ecosystems, the various habitats are populated by various bacteria, each of them adapted to their respective living environment. For instance, dry skin areas such as the forearm host a large number of different species (Costello *et al.* 2009, Grice *et al.* 2009). By contrast, the bacterial variety is less in the sebaceous regions. Typical representatives of bacteria inhabiting the sebaceous and sweat-rich body regions are the species *Staphylococcus spp*, *Corynebacterium spp* and *Proteobacteria* (Callewaert *et al.* 2013). The skin microbiome comprises four phyla in the main: *Actinobacteria, Firmicutes*, *Bacteriodetes* and *Proteobacteria* (Grice and Segre 2012). Biodiversity is correspondingly high, as each phylum consists of several thousands of species, each specifically adapted (Costello *et al.* 2009, Findley and Grice 2014, Grice *et al.* 2009). The interspecific diversity of the affected skin areas between various individuals is greater than the intraspecific diversity (Gao *et al.* 2007).

1.2.2 Development of the individual microbial fingerprint

Depending on the type of childbirth delivery, the microbial inhabitation of humans begins immediately after birth, primarily through the maternal microbiome around the birth canal, as well as through close skin contact after birth (Dominguez-Bello *et al.* 2010, Sarkany and Gaylarde 1968). In the ensuing years up to the 4th year of life, a stable core microbiome is developed (Capone *et al.* 2011) which is of great significance for human health. A lack of this permanent microbial inhabitation leads to increased infestation with pathogens, which results in long-term skin damage, susceptibility to diseases and the development of allergies (Lynch 2016, Riiser 2015). The human skin microbiome provides protection against infestation with foreign bacteria and influences the human immune system. Studies confirm that *Staphylococcus epidermidis*, a typical skin commensal, secretes the protease Esp which inhibits biofilm formation of the disease pathogen *Staphylococcus aureus* in the nose (Iwase *et al.* 2010). Moreover, skin-inhabiting staphylococci produce antimicrobial peptides (AMPs) which are specifically designed to protect against pathogenic infestation. These AMPs are phenol-soluble

modulins which interact with bacterial membrane lipids resulting in the formation of complexes. These AMP-lipid complexes are responsible for the antimicrobial effect. *S. epidermidis* communicate with the host via δ-toxins under the induction of host-specific AMPs, such as the cathelicidin LL-37, which is part of the innate human immune response (Cogen *et al.* 2010, Tomasinsig and Zanetti 2005).

The development of a core microbiome which remains stable over time (Ding and Schloss 2014) is distinct and is the equivalent to a bacterial fingerprint (Fierer *et al.* 2010, Grice *et al.* 2009). The development of the human microbiome depends on many different factors. The specification of the human intestinal microbiome occurs through the type of nutrition immediately after birth (breastfeeding or infant formula) (Bergstrom *et al.* 2014, Jost *et al.* 2015), as well as the introduction of complementary feeding between the 6^{th} and 24^{th} months of life (Thompson *et al.* 2015). The concomitant change in the availability of nutrients for the intestinal bacteria determines the composition of the microbiome of the gastrointestinal tract (Tremaroli and Backhed 2012, Turnbaugh and Gordon 2009). An increased carbohydrate and protein-rich diet favours *Bacteriodetes* species, whereas *Prevotella spp* thrive when fruit and vegetables are the main nutrient source (De Filippo *et al.* 2010, Shankar *et al.* 2017). In addition, there are mainly sex-specific differences in the variation of the microbiome caused by the differing hormonal configuration and different mode of life (Fierer *et al.* 2008, Giacomoni *et al.* 2009). With the increased use of cosmetic products such as make-up by the female sex, greater diversity of the microbiome in the affected skin areas can be observed due to the application of additional nutrients to the skin (Staudinger *et al.* 2011). In addition to sex-specific differences in hormone production, stress and natural ageing processes in humans (Leyden *et al.* 1975, Merchant *et al.* 2016, Somerville 1969) lead to a shift in the hormone balance which in turn results in changes to the composition and secretion of sweat and sebum, which constitute the basic food resource of a wide variety of microorganisms on the skin (Callewaert *et al.* 2014, Fierer *et al.* 2008, Giacomoni *et al.* 2009, Marples 1982). Moreover, different geographies of human populations influence the makeup of the microbiome. People who live in warm and humid regions tend to host a greater bacterial diversity than people who live in cold, dry areas (Lloyd-Price *et al.* 2016, The human microbiome project consortium 2012). Exposure to UV light is yet another decisive factor, as UVB radiation inhibits bacterial growth (Faergemann and Larko 1987).

In addition to the permanent core microbiome, there are bacteria which colonise the human body for only short periods (Ellis 1981) and which are highly dependent on the living conditions

of each respective host and his or her environment. Daily exposure to contaminants or pathogens initially affects the transient microbiome and subsequently the permanent makeup of the microbiome as well (Price 1938). This includes increased exposure to xenobiotic contaminants in urbanised areas.

The use of antibiotics also has a great influence on the development and composition of the human microbiome. These anti-infective substances unfold their bactericidal effect not only on pathogens, they also attack the microbiome. Studies have shown that the regeneration of the microbiome is often protracted and can result in an altered microbiome composition (Dethlefsen *et al.* 2008, ElRakaiby *et al.* 2014, Leclercq *et al.* 2017). Stool samples from children treated with antibiotics showed a significant reduction in the numbers of bacterial populations as well as changes in the composition of their microbiome (Palmer *et al.* 2007). Whereas the bacterial composition of the microbiome in children is continuously re-established over time, in adults it results in a long-term loss of the microbial composition. Accordingly, certain microbial taxa were no longer detected in adult study participants 6 months after treatment with the antibiotic ciprofloxacin (Dethlefsen *et al.* 2008).

1.2.3 The interrelationship between the host and the microbiome

The human body and the microbiome have a mutualistic interrelationship whereby both take advantage from this symbiosis (Backhed *et al.* 2005). Through the excretion of sweat and sebum and the intake of various nutrients, humans provide a wide variety of habitats on surfaces with many different structures (Devaraj *et al.* 2013, Scharschmidt and Fischbach 2013, Schommer and Gallo 2013). In return, the intestinal microbiome produces essential vitamins and amino acids, such as the fat-soluble vitamin K (Nicholson *et al.* 2012). Moreover, human epithelial cells produce and excrete specific AMPs, such as cathelicidins and defensins, which protect the skin from colonisation by foreign pathogens and serve to protect the predominant microbiome of the host (Gallo *et al.* 2002). Furthermore, communication between the microbiome and the host as well as the activation of the human immune system take place via various cytokines, chemokines and proteases (Christensen and Bruggemann 2014).

In addition to the communication of the individual bacteria species among one another via mechanisms such as *quorum sensing* (Braga *et al*. 2016, Szafranski *et al.* 2017), and the production of various AMPs and signalling via biofilms (Beatty *et al.* 2017, Wang *et al.* 2017),

there is far-reaching interspecific communication between the microbiome and the host. To communicate with the host, the microbiome uses a variety of signal molecules ranging from lactones, indoles and peptides through polysaccharide and short-chain fatty acids (SCFA) to lipids (Rath and Dorrestein 2012). The endocrine activity of the intestinal microbiota includes the production and regulation of SCFA as well as neurotransmitters such as ɤ-aminobutyric acid (GABA) or acetylcholine, bile acids, testosterone, cortisol and gastrointestinal hormones (Cani *et al.* 2013, Clarke *et al.* 2014). These molecules can pass the host's blood-brain barrier (Cryan and O'Mahony 2011). In addition to their role as messenger molecules, they are also able to influence host's behaviour (Martin and Mayer 2017, Montiel-Castro *et al.* 2013). For instance, in social studies mice which had been fed with the probiotic culture *Lactobacillus rhamnosus* showed diminished fear behaviour and a reduced stress level which can be linked to the concomitant increased expression of the GABA receptor (Bravo *et al.* 2011). Intestinal bacteria and probiotic cultures can determine the level of cytokines through which the parasympathetic system of the host is proven to be influenced (Forsythe and Bienenstock 2010). There are also more and more indications that the intestinal microbiome may be the trigger of various neuronal diseases such as autism. Autistic mice show defects in the barrier of the gastrointestinal tract as well as a modified intestinal microbiome. Mice which were given *Bacteroides fragilis* cultures showed a correction of the permeabiliy of the intestinal wall and a more stable microbiota and also possessed improved communicative abilities (Hsiao *et al.* 2013).

1.2.4 Xenobiotic metabolism of the human microbiome

On the basis of metagenome sequencing, Qin *et al*. published a set of sequencing data that suggests that intestinal bacteria are capable of degrading xenobiotic substances such as aromatics (Qin *et al.* 2010). Under the precondition of a consistent exposure to certain substances and substance classes, bacteria tend to maintain enzymes necessary for substance metabolisation or detoxification. With regard to the conserved sequences found in the gut metagenome consistent exposure to the corresponding xenobiotic aromatic substances can thus be assumed. At 37° C the intestinal bacterium *Escherichia coli* has an average doubling time of 20 minutes. This is the temperature which complies with the natural milieu in the human gut (Freter and Ozawa 1963). The entire genome is replicated once and passed on to the daughter cell. The high division rate causes faster mutation rates during DNA replication while conjugation processes also take place which enable the bacteria to exchange their genome

partially or completely among one another. Genetic material is transferred, which gives the bacteria a selective advantage within their environment. Enzymes which are not constantly required for bacterial metabolism, communication and defence are lost through DNA replacement and higher mutation rates in the form of deletions or the loss of entire operon structures.

A large variety of medications can be metabolised by the human intestinal microbiome (Jourova *et al.* 2016, Nichols *et al.* 2016, Wilson and Nicholson 2017). The intestinal bacterium *Eggerthella lenta* can inactivate the effects of digoxin, which is used mainly to treat heart failure, by reducing the double bond on the lactone ring (Haiser *et al.* 2013). Another example is levodopa (also known as L-Dopa or L-3,4-dihydroxyphenylalanine), which is used as a prodrug in the treatment of Parkinson's disease. Unlike its activated component dopamine, levodopa can overcome the human blood-brain barrier and be converted in the brain into the medically active form of dopamine. By decarboxylation by the bacterium *Helicobacter pylori*, levodopa is converted into dopamine in the intestinal tract. The resulting dopamine cannot pass the blood-brain barrier and hence cannot unfold its effect in the basal ganglia (Pierantozzi *et al.* 2006).

Because of the chemical conversion of the administered substances, the microbiome has a decisive influence on the effect of a variety of medications (Enright *et al.* 2016). However, the breakdown of xenobiotic substances can also lead to an inactivation of hazardous materials. In contrast, negative effects for humans may arise through the metabolisation of primary contaminants into highly reactive substances (Tralau *et al.* 2015). The latter has been examined in detail in this thesis for the human skin microbiome.

1.3 Polycyclic Aromatic Hydrocarbons (PAHs)

1.3.1 Chemical properties of polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are organic hydrocarbon compounds consisting of at least two condensed benzene ring systems (Sims and Overcash 1983). Based on the number of condensed aromatic ring systems, low molecular weight (LMW) PAHs, which consist of two or three condensed benzene rings, and high molecular weight (HMW) PAHs, which are composed of at least four annulated aromatic hydrocarbon rings are distinguished (Figure 1)

(Feng *et al.* 2009, Mackay and Callcott 1998). In addition, the aromatic rings can be substituted, in particular by methyl and oxygen groups, resulting in a large variety of different PAHs (Fetzer 2007). Approximately 300 known compounds are categorised as PAHs. *Ortho*-annulated systems feature a linear arrangement of aromatic benzene rings. In contrast, the term *peri*-annulated compounds refers to PAHs with aromatic ring systems in an angled arrangement (Bamforth and Singleton 2005).

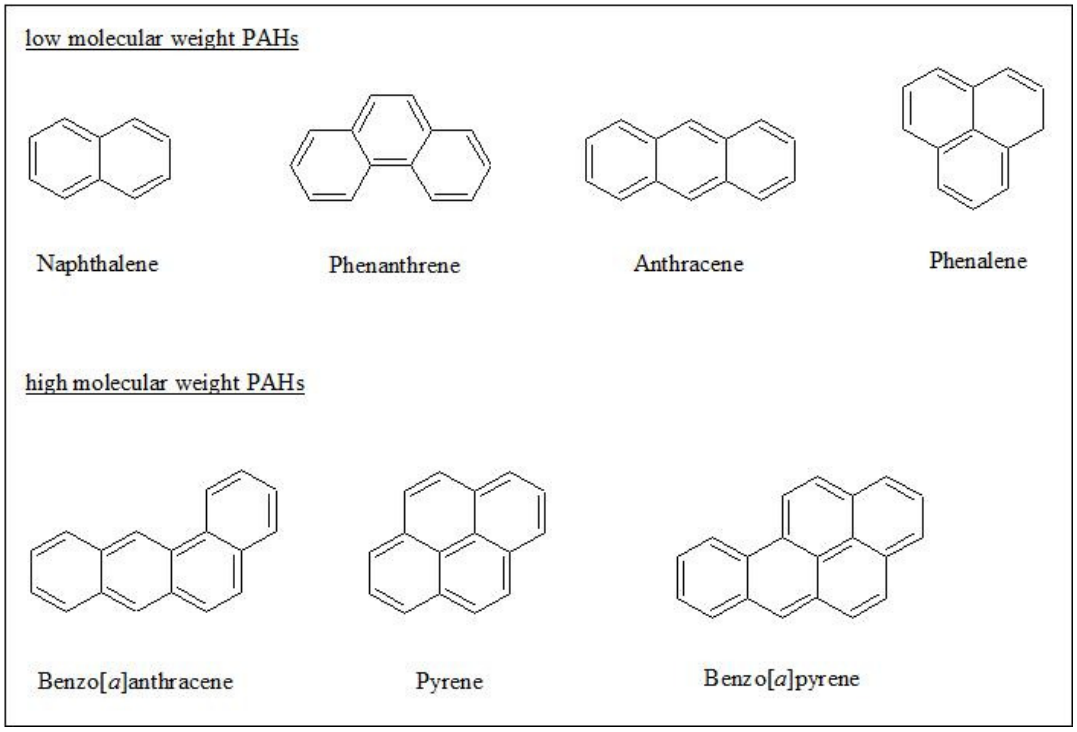


Fig. 1 Illustration of selected polycyclic aromatic hydrocarbons.

The polarity of the PAHs, and thus also their solubility in aqueous environments, decreases with an increasing number of aromatic ring systems (Cerniglia 1992). For this reason, primarily the high molecular weight PAHs exhibit a high tendency towards geoaccumulation (Danyi *et al.* 2009, Feng *et al.* 2009, Ishizaki *et al.* 2010). The lipophilicity of PAHs results in good solubility in organic solvents (Juhasz and Naidu 2000). In their pure form, most cyclic aromatic systems are crystalline, usually colourless solids. Analytical determination of these substances is facilitated by the auto-fluorescence property possessed by various PAHs such as benzo[*a*]pyrene (B[*a*]P). In the 1980s, the US EPA selected 16 PAHs as lead substances for environmental analyses (Keith 2015). Naphthalene, acenaphthylene, acenaphthene, fluorene,

anthracene, benzo[*a*]anthracene, fluoranthene, pyrene, phenanthrene, chrysene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, benzo[*k*]fluoranthene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a*,*h*]anthracene and benzo[*g*,*h*,*i*]perylene from diverse sample materials were analysed as representatives of the large group of PAHs.

1.3.2 Origin, occurrence and exposure of PAHs

Generally speaking PAHs result from incomplete biotic and abiotic combustion processes of organic materials (Ramesh *et al.* 2011). The main sources of PAHs are from industrial processes in petrochemistry, coking plants, aluminium production, iron and steel production, and power plants (Boffetta *et al.* 1997). Other significant anthropogenic sources of PAHs include industrial combustion processes of fossil fuels (Commins 1969, Mohn *et al.* 1997) and the use of combustion engines. Biotic entry of PAHs into the environment occurs primarily as a result of forest and bush fires as well as volcanic eruptions (Budavari 1989, Morillo *et al.* 2007).

In inoperative industrial plants and old coking plants, high contamination with substances damaging to the environment and human health, including PAHs, is generally found in soils (Juhasz and Naidu 2000). The distribution of these substances is inhomogeneous and depends on the substances used and produced. Consortia of fungi and bacteria are able to degrade these contaminants and use them as carbon and/or energy sources (see Section 1.5) (Cerniglia 1992, Haritash and Kaushik 2009, Juhasz *et al.* 1996).

Exposure of humans to PAHs takes place mainly via waste gases from industry and road traffic. The aromatic systems are also released during the preparation of food, for example when barbecuing and curing (Ramesh *et al.* 2011, Schröder 2010) as well as in the form of tobacco smoke (Lee *et al.* 1976, Sepetdjian *et al.* 2008). In addition PAHs are natural components of extender oils and carbon blacks that are added during manufacturing processes in order to achieve desired product properties. Extender oils are used, for example, as additives in the production of smooth rubber coatings, while carbon blacks are primarily added to elastomers in order to positively affect the elastic and attenuation properties of the products. Relatively large amounts of extender oils in particular have been detected in children's toys (statement of the BfR 051/2009). Other contaminated consumer products include the rubber coatings of tool handles, bicycle handlebars, beach sandals and wristwatches (statement of the BfR 025/2009, Tarnow *et al.* 2016). Daily dermal contact may result in migration of the substances into skin

and thus presents a potential risk to human health (statement of the BfR 032/2010, Biedermann and Grob 2010, statement of the EFSA 2008, Grob *et al.* 1991).

Many PAHs, particularly the high molecular weight PAHs, are highly carcinogenic and mutagenic following enzymatic activation through monooxygenases and dioxygenases (see Section 1.4 and Section 1.5) (Xue and Warshawsky 2005). Furthermore, activated PAHs may function as tumour promoters. Although the aromatic compounds themselves are not intrinsically carcinogenic, they promote or strengthen the cancer-inducing effects of other substances (Levin *et al.* 1986).

1.4 Metabolism of B[*a*]P in human cells

The metabolic activation/detoxification of polycyclic aromatic hydrocarbons has been thoroughly investigated in eukaryotic cells (Baird *et al.* 2005, Luch 2009, Shimada 2006), the aromatic five-ring system B[*a*]P being a model substance. It is a procarcinogen because it has a carcinogenic effect only after activation of the five-ring aromatic hydrocarbon. Initial activation is possible in three ways. In addition to radical oxidation through peroxidase reactions and the *ortho*-quinone pathway, the oxidation reaction mediated by cytochrome P450 monooxygenases (CYP) is significant. This is the starting point for the occurrence of the carcinogen benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE) (Luch 2005, Shimada 2006).

During radical oxidation, a radical cation is formed through one-electron oxidation mediated by a peroxidase (Jiang *et al.* 2007). This radical cation can interact with cellular DNA, resulting in the formation of unstable, depurinated B[*a*]P-cation·DNA adducts (Baird *et al.* 2005, Cavalieri *et al.* 2005).

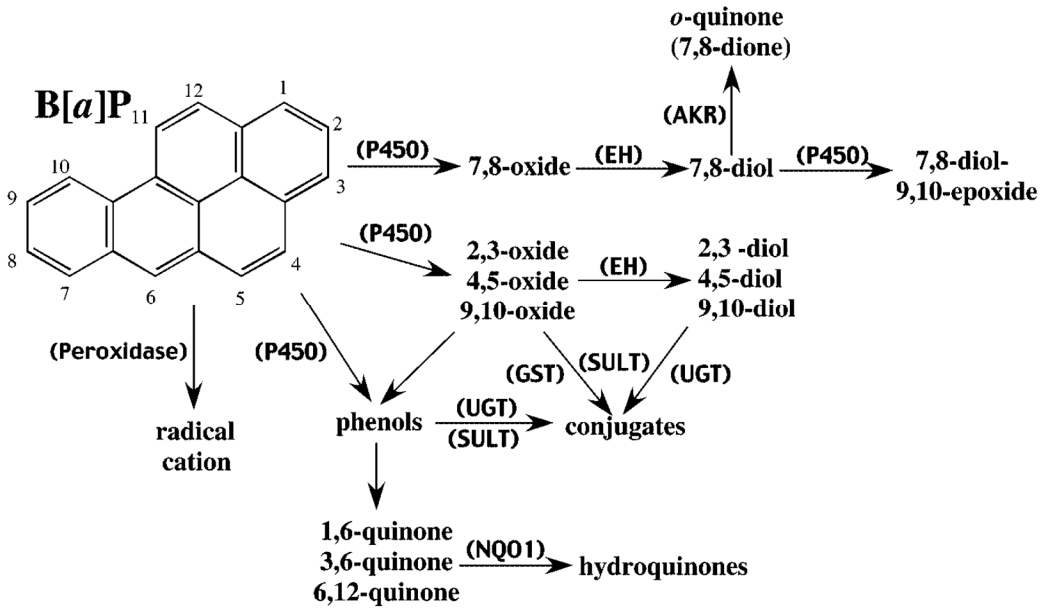


Fig. 2 Activation and metabolisation of benzo[*a*]pyrene in eukaryotic cells according to Shimada 2006.
(P450 cytochrome P450; EH epoxide hydrolase; AKR aldo-keto reductase; SULT sulfotransferase; UGT UDP-glucuronosyltransferase; GST glutathione-S-transferase; NQO1 NAD(P)H quinone oxidoreductase)

The *ortho*-quinone pathway begins with initial oxidation through CYPs (Shimada and Fujii-Kuriyama 2004). The starting point is phenolic B[*a*]P derivates, which can be converted to B[*a*]P-*o*-quinones through the formation of catechols in a reaction mediated by aldo-keto reductase (Palackal *et al.* 2001). Quinones are reactive compounds (electrophiles) that act with the DNA under formation of stable, depurinated DNA adducts. Via phase II reactions, the quinone derivates can be detoxified and finally eliminated with the help of sulfotransferases, glucuronosyltransferases or glutathione-S-transferases (Hecht 2002). The different activation pathways of B[*a*]P are summarised in Figure 2.

The best-known reaction pathway to activation of B[*a*]P results in the formation of the carcinogen BPDE (Conney 1982). The initial oxidation is mediated by CYPs (Bauer *et al.* 1995, Oesch *et al.* 2014). The resulting B[*a*]P dioxides are hydrolysed by microsomal epoxide hydrolases. B[*a*]P dihydrodiols are formed which are less reactive but polar. These electrophiles can interact with substances such as glucuronides, mercapturic acid, acetate, methyl or sulphate groups, thiocyanates or glucosides in phase II reactions through conjugation processes. This results in excretable hydrophilic products enabling detoxification of the hydrophobic and toxic original substance (Nebert and Dalton 2006, Shimada 2006, Williams 1971). B[*a*]P diols can be further converted through CYP-mediated oxidation, resulting in BPDE. This exogenous electrophile can interact with endogenous nucleophiles such as the adenine and guanine

residues of the DNA. Stable DNA adducts are formed, for example on the second nitrogen atom of the guanine. The carcinogenic effect is based on the alkylation of DNA by a carbonium ion derived from the B[*a*]P epoxide. Formation of the carbonium ion is facilitated if it is part of the so-called bay region. The bay region is characteristic of PAHs with carcinogenic potential and represents a "bay" between three annulated benzene rings with elevated electron density. A stable BPDE desoxyguanosine adduct is formed by the electrophilic attack of the carbonium ion of the B[*a*]P epoxide on the second nitrogen atom of the guanine. The reaction of the BPDE with the DNA bases results in the formation of lesions in the DNA double helix. Furthermore, chromosomal aberrations and sister chromatid exchange can occur (Hollstein *et al.* 1979). The form of mutations due to DNA damage depends on the location of the modification and on the adjacent nucleotides, while the cell has various DNA repair mechanisms that can detect and correct such DNA damage (Brinkmann *et al.* 2013). The p53 tumour suppressor protein is a key factor in the activation and execution of DNA repair mechanisms. DNA adducts can be removed via the nucleotide excision repair mediated by p53 (Fitch *et al.* 2003, Nelson and Kastan 1994). In the event of irreparable DNA damage, pro-apoptotic signals are transmitted by p53 which lead to the activation of the intrinsic apoptosis pathway and thereby to cell death (Kastan *et al.* 1992, Nelson and Kastan 1994).

The monooxygenases CYP1A1 and CYP1B1 play a major role in the initial activation of B[*a*]P (Kim *et al.* 1998). After eukaryotic cells come into contact with PAHs such as B[*a*]P or dioxins both *CYP1A1* and *CYP1B1* are transcriptionally induced (Oesch *et al.* 2014). The B[*a*]P-induced *CYP* gene expression is mediated by the aryl hydrocarbon receptor (AHR) (Burczynski and Penning 2000).

The AHR is a transcription factor that belongs to the basic-helix-loop-helix (bHLH)-Per-Arnt-Sim (PAS) family and has a broad range of physiological functions in immune response, apoptosis, skin differentiation, endocrine signalling, and host-microbiome communication (Bessede *et al.* 2014, Esser *et al.* 2009, Fernandez-Salguero *et al.* 1997, Murray *et al.* 2014, Ohtake *et al.* 2003, Stolpmann *et al.* 2012, Tarnow *et al.* 2016, Tralau *et al.* 2015, Veldhoen *et al.* 2008). Moreover, as a signal transduction protein and transcription factor, the AHR has a significant influence on both the activation of the procarcinogen B[*a*]P and the further metabolisation of B[*a*]P derivates in eukaryotic cells. This means that the AHR is one of the main regulators of physiological and xenobiotic metabolic processes in eukaryotic cells (Barouki *et al.* 2012, Bauer *et al.* 1995, Gasiewicz *et al.* 2008). The receptor moves continuously between the nucleus and cytoplasm (Richter *et al.* 2001). Depending on the ligand,

activation of the receptor occurs after association with xenobiotics such as B[*a*]P or β-naphthoflavone (β-NF). A simplified model according to Lees and Whitelaw, 1999 indicates that the cytoplasmic complex, consisting of the AHR and the chaperone Hsp90 (Perdew 1988), protein p23 (prostaglandin E synthase 3) and the immunophilin-like AHR-interacting protein (better known as hepatitis B virus X-associated protein 2 (XAP2)) (Meyer *et al.* 1998) dissociates after association with ligands. The AHR translocates to the nucleus (Denison *et al.* 2011), where it assembles into a heterodimer with the nuclear translocation protein ARNT (Probst *et al.* 1993, Reyes *et al.* 1992). The AHR-ARNT complex connects to xenobiotic response elements (XREs) in the promoters of target genes such as CYPs, where it induces gene expression as a transcription factor (Hahn *et al.* 2009, Ikuta *et al.* 1998, Ko *et al.* 1997). While nucleocytoplasmatic shuttling of the AHR and its importance for maintaining a cytoplasmic surplus of this protein have been recognised as early as 1998, the underlying molecular mechanisms remained largely unknown. The targeted, ligand-induced transport of the AHR to the cell nucleus is significantly faster than the constitutive, ligand-independent translocation. The constitutive AHR import and export, as well as the activation of the receptor are determined by two areas of the N-terminal domain of the AHR, depending on specific ligands. The regulation of the import of the AHR complex is initiated by the nuclear localisation signal (NLS) (Ikuta *et al.* 1998). This process takes place both depending on and independently of exposed ligands. In addition, the NLS domain is important for ligand-independent export of the AHR from the cell nucleus into the cytoplasm. The nuclear export signal (NES) domain encompasses the N-terminal amino acid region 63 – 73 and shows significant sequence similarities with mammalian DNA motifs. This domain overlaps with regions that interact with the nuclear translocation protein ARNT. It is therefore assumed that masking of the NES domain through ARNT blocks the export of the AHR from the cell nucleus, whereby the receptor complex can interact with XREs. The NES domain exclusively regulates the export of the AHR from the cell nucleus (Davarinos and Pollenz 1999), but needs additional structural elements of the C-terminal domain for this purpose that are not yet known. This is caused by the exchange of the C-terminal domain of the human AHR for the murine construct resulting in an accumulation of the AHR in the nucleus (Ramadoss and Perdew 2005). Export from the nucleus results in ceasing of transcription of target genes such as CYPs and the promotion of proteasomal degradation of AHR.

1.5 Biological degradation of geoaccumulated polycyclic aromatic hydrocarbons in environmental microbiology

1.5.1 Microbial isolates from soil microbiology and their potential to metabolise HMW PAHs

Entry of PAHs into the environment occurs constantly, leading to their presence in air, stone, soil and sediments (Juhasz and Naidu 2000). Through abiotic processes such as photolysis and chemical oxidation, gradual degradation of the harmful substances takes place and evaporation of small quantities prevents the accumulation of LMW PAHs (Juhasz and Naidu 2000). These processes are extremely slow, and only a small proportion of the PAHs is removed from the environment. Particularly the high molecular weight PAHs accumulate in the soil layers. Due to their chemical durability, they pose a problem in highly polluted areas of old industrial facilities (Mackay and Callcott 1998, Morillo *et al.* 2007). The high hydrophobicity of the HMW PAHs and their ability to adhere firmly to sediments and inorganic material results in low acute bioavailability (Haritash and Kaushik 2009).

Biological degradation of PAHs primarily takes place through bacterial consortia together with various fungi. It is therefore difficult to predict the composition and type of consortia as well as the specific degradation pathway and possible intermediates (Meulenberg *et al.* 1997, Silva *et al.* 2009). Typical bacterial representatives involved in PAH degradation belong to the genera *Pseudomonas*, *Mycobacteria*, *Rhodococcus* and *Sphingomonas* (Aitken *et al.* 1998, Haritash and Kaushik 2009). Fungi involved in the degradation primarily derive from the group of ligninolytic fungi. In principle, three genera have been described: *Basidiomycetes*, *White rot fungi* and *Deuteromycetes* (Harms *et al.* 2011). Fungi usually perform the first metabolic step to PAH degradation (Meulenberg *et al.* 1997). There are two possibilities. (1) Through the cytochrome P450 system, hydroxylated PAH metabolites are formed in a similar way to the metabolism in mammals (Yadav *et al.* 2006). In the first step, the CYP-dependent monooxygenase is activated and PAHs are converted to *trans*-dihydrodiols through oxygen entry and then through epoxide hydrolases (Cajthaml *et al.* 2002, Cerniglia 1997). (2) The second possibility is initial degradation through extracellular enzymes of ligninolytic fungi (Pointing 2001). These enzymes include lignin peroxidase, manganese-dependent peroxidase, and phenoloxidases, which mainly utilise lignocellulose in their environment (Gianfreda *et al.* 1999, Steffen *et al.* 2003). These enzymes perform one-electron oxidation whereby cation

radicals are created. These can be more quickly and easily taken up and metabolised by bacteria than the hydrophobic lead compounds (Vyas *et al.* 1994). However, because lignins have different structures, the enzymes have predominately low substrate specificity. Thus, Hofrichter *et al*. could demonstrate that the manganese peroxidase isolated from the white rot fungus *Nematoloma frowardii* is able to partially convert various aliphatic and aromatic hydrocarbon compounds. These include, for example, pentachlorophenol, catechol, tyrosine, tryptophan, pyrene, 2-amino-4,6-dinitrotoluene, as well as the aliphatic compounds urea, aspartate, glutamate, fructose, glucose, glyoxylate aldehyde, glycine and leucine (Hofrichter *et al.* 1998). Ligninolytic fungi also convert PAHs through the same process (Cajthaml *et al.* 2002, Haritash and Kaushik 2009, Peng *et al.* 2008, Vyas *et al.* 1994).

Microbial degradation of PAHs can take place in a fermentative or respiratory manner by using molecular oxygen as an electron acceptor (Baboshin and Golovleva 2012, Chang *et al.* 2008, Tsai *et al.* 2009). Anaerobic degradation is performed by sulphate- and nitrate-reducing bacteria. The reduction process is directly linked to the biotransformation of PAHs. In comparison with aerobic degradation, several consecutive hydrations and hydrolysis reaction steps take place initially. By means of a concluding decarboxylation step, the central intermediate phenol is formed from, for example, phenanthrene and is then split during final ring cleavage. Activation takes place through hydrolysis and carboxylation reactions. The end products, acetic acid and water, are finally formed through the gradual degradation of the aromatic hydrocarbon (Tsai *et al.* 2009).

Aerobic bacterial degradation of PAHs is usually initiated by dioxygenases, but sometimes also by monooxygenases. Here, molecular oxygen is required for the initial enzymatic attack of the benzene rings, which ultimately results in the formation of *vicinal* dihydrodiols. The subsequent ring cleavage takes place by means of intradiol or extradiol ring cleavage dioxygenases. Aromatic degradation proceeds via continuous dehydrogenation and decarboxylation. Thereafter, another attack of a dioxygenase takes place in order to form dihydrodiols. The next ring cleavage is prepared and the degradation of the aromatic system finally takes place according to the described mechanism. Final ring cleavage reactions occur with the last remaining benzene ring. In this process, a catechol derivate is formed by means of an NAD(P)H-dependent dehydrogenase which results in two different intermediate products during the ring cleavage in the *ortho* or *meta* direction: muconic acid (*ortho* cleavage) or 2-OH-muconic acid semialdehyde derivate (*meta* cleavage). The cleaved rings are degraded in simple β-oxidation processes whereby the components enter the tricarboxylic acid cycle as activated

3-oxoadipate and are used for producing energy and bacterial biomass (Cerniglia 1992, Eaton and Chapman 1992).

Bacterial degradation of high molecular weight PAHs has only been described for a small number of environmental bacteria, particularly with regard to use as sole source of carbon and energy. These include *Mycobacterium vanbaalenii* PYR-1 (Heitkamp *et al.* 1988, Khan *et al.* 2002) and *Sphingomonas paucimobilis (*Gibson *et al.* 1975). Other additives are usually needed to initiate the metabolic pathway, e.g. additional LMW PAHs (Yuan *et al.* 2002) or sugars such as succinate, glucose or pyruvate (Chang *et al.* 2008, Lee *et al.* 2003, Ye *et al.* 1996). *S. paucimobilis* is the first bacterium for which the degradation of B[*a*]P using the identified metabolites B[*a*]P-9,10-dihydrodiol and B[*a*]P-7,8-dihydrodiol (Gibson *et al.* 1975, Pinyakong *et al.* 2003) has been described. However, no ring cleavage products have been identified that could provide direct indications of the route of degradation.

The respective initial dioxygenases for the degradation of LMW PAHs have been thoroughly studied (Brezna *et al.* 2003, Haritash and Kaushik 2009, Khan *et al.* 2001, Peng *et al.* 2008). The multi-component system is composed of an NAD(P)H oxidoreductase, a ferredoxin component and an oxygenase component that contains the active side (Brezna *et al.* 2003, Brezna *et al.* 2006). The catalytic proportion of the dioxygenase consists of small α- and large β-subunits (SU). The α-SU contains Rieske iron-sulphur clusters and mononuclear non-haem iron. Two electrons of the reduced pyridine ring of the NAD(P)H are transferred via the reductase, the Rieske protein, to the catalytically active iron (Peng *et al.* 2008). These reducing elements allow the activation of molecular oxygen, which can be used to attack the aromatic nucleus of the PAHs (Khan *et al.* 2001, Kim *et al.* 2006, Kweon *et al.* 2008). This enzyme has been detected in *Pseudomonas putida* on plasmid p*NAH7* (Yen and Gunsalus 1982, Yen and Gunsalus 1985). *P. putida* is able to metabolise two-ring systems such as naphthalene in particular, but also the three-ring systems phenanthrene and anthracene. The catabolic genes of the naphthalene degradation are arranged into two operon structures, whereby the first operon contains the genes for enzymes for conversion of naphthalene into salicylate. Genes on the second operon encode enzymes that convert salicylate into pyruvate and acetyl-CoA. These metabolites are fed directly into the tricarboxylic acid cycle (Yen and Gunsalus 1982, Yen and Gunsalus 1985). In addition to catabolic plasmids, bacteria whose route of degradation is coded in the genomic DNA also exist, e.g. *Mycobacterium vanbaalenii* PYR-1 (see Section 1.5.5) (Kim *et al.* 2008).

There has been far less research into the dioxygenase system for the degradation of high molecular weight PAHs such as B[*a*]P. However, it is known that *M. vanbaalenii* PYR-1 requires 27 enzymes for the complete degradation of pyrene, including an initial dioxygenase (Kim *et al.* 2004, Kim *et al.* 2008, Kim *et al.* 2005, Kweon *et al.* 2015, Kweon *et al.* 2011).

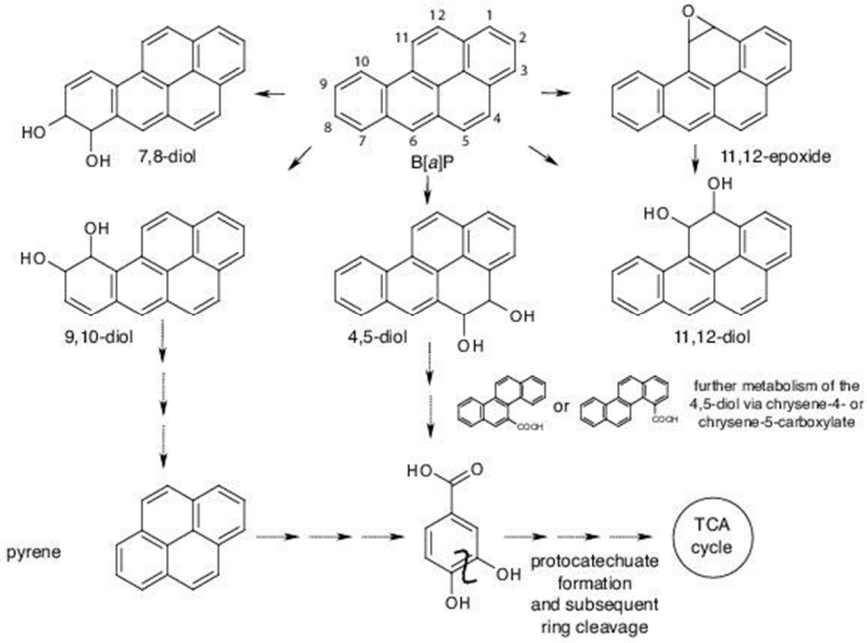


Fig. 3 Prokaryotic activation of benzo[*a*]pyrene adapted from Sowada *et al*., 2013.
The metabolisation is usually based on the reaction of initial dioxygenases that result in the formation of dihydrodiols, which are then metabolised further.

1.5.2 Biotic and abiotic factors that influence microbial PAH degradation

The microbial degradation of PAHs can take place under anaerobic conditions, i.e. without oxygen, or aerobic conditions. Aerobic degradation follows the use of oxygen as an electron acceptor in the respiratory chain. In contrast, under anaerobic conditions inorganic substances such as nitrate, sulphate or Fe(III) are used as electron acceptors (Anderson and Lovley 1999, Tsai *et al.* 2009). In this way, the bioavailability of inorganic compounds determines the potential for anaerobic PAH degradation. Under sulphate- or nitrate-reducing conditions, PAHs are converted to low molecular weight acids. These acids can then act as ligands and chelate insoluble Fe(III)oxide. The activated Fe(III)oxide is thus available to Fe-reducing bacteria, which further intensifies the degradation of PAHs (Schmitt *et al.* 1996). Consequently, in

addition to the availability of inorganic substances, the formation of consortia is also a decisive biotic factor in PAH metabolisation. Under anaerobic conditions, sulphate-reducing bacteria, for example, act together with methanogenic microorganisms as a consortium (Chang *et al.* 2002). Consortia of fungi and bacteria are also highly prevalent. The fungi, such as white rot fungi, excrete extracellular enzymes, e.g. lignin peroxidase, manganese-dependent peroxidase and phenoloxidase, which catalyse the initial ring attack of PAHs and which simultaneously constitute the limiting step (Clemente *et al.* 2001, Hofrichter *et al.* 1998). The bioavailability to bacterial cultures is thereby increased and (membrane-) toxic original substances are already detoxified (Juhasz and Naidu 2000). Another possibility is the formation of consortia with bacteria which produce surface-active substances such as *Pseudomonas aeruginosa*. The secreted surfactants solubilise sedimented PAHs. This increases the bioavailability of hydrophobic PAHs that are difficult to access (Chang *et al.* 2015, Mueller *et al.* 1989).

Originating from contaminated industrial areas with a large number of different xenobiotic components and PAH mixes, the presence of additional co-metabolites has a significant influence on the success of microbial PAH degradation. The availability of different co-metabolites increases the PAH degradation rate and/or initiates the degradation of high molecular weight PAHs. These co-metabolites include mixes of different PAHs on the one hand (Stringfellow and Aitken 1995) and simple organic acids, such as pyruvate or lactate on the other (Lee *et al.* 2003). In the presence of these substances, the metabolic conversion of PAHs such as phenanthrene, pyrene and B[*a*]P is verifiably increased. The additional hydrocarbon serves as an initial bacterial growth stimulant and is also used for initial enzyme activation of the metabolic PAH conversion (Chang *et al.* 2008, Yuan *et al.* 2002).

The degradation of LMW PAHs such as naphthalene or phenanthrene is faster than the degradation of PAHs with more than four aromatic ring systems (Johnsen *et al.* 2005). This is primarily due to the bioavailability of HMW PAHs, the uptake of these high molecular weight substances via the bacterial membrane, and the enzymatic activity of the PAH degradation. The chemical structure of polycyclic aromatic hydrocarbons determines their solubility in water and their sedimentation potential (Haritash and Kaushik 2009). PAHs with up to three annulated benzene rings have a lower n-octanol/water partition coefficient. This means that they have better hydrophilicity than HMW PAHs and are therefore more bioavailable than PAHs with four or more annulated benzene ring systems (Juhasz and Naidu 2000). Bioavailability increases with rising temperature by increasing the desorption rate of PAHs that are firmly adsorbed on or in sediments and soil particles (He *et al.* 1995, Lüers and ten Hulscher 1996).

With the rise in temperature, the metabolic activity of the microorganisms and the optimum activity of required enzymes also increase. Weissenfels *et al.* showed that maximum PAH oxidation rates for fluorene, fluoranthene and phenanthrene were achieved at a temperature of 30° C and with a pH-value in the region of 7.0 (Weissenfels *et al.* 1990). A rise in temperature to over 40° C led to an increase in the membrane toxicity of the hydrocarbons and thus to a reduction in bacterial PAH metabolisation (Leahy and Colwell 1990). Kästner *et al.* showed that shifting the pH value into the neutral range resulted in the activation of *sphingomonads* – typical bacterial representatives of PAH degradation – which then metabolised phenanthrene (Kastner *et al.* 1998). In contrast, *M. vanbaalenii* PYR-1 seems to achieve a better degradation rate in acidic environments. This is probably linked to increased permeability of the outer membrane (Kim *et al.* 2005).

1.5.3 The model organism *Mycobacterium vanbaalenii* PYR-1

With respect to PAH degradation, the most well studied bacterium is *M. vanbaalenii* PYR-1. This bacterium, belonging to the *Actinobacteria* group, was originally isolated from soil samples contaminated with oil and is considered the first bacterium proven to be able to use HMW PAHs as the sole carbon and energy source (Heitkamp and Cerniglia 1988, Khan *et al.* 2002). Mycobacteria have a lipophilic surface, which means that this group of bacteria is predestined for taking up lipophilic contaminants from soil particles (Haritash and Kaushik 2009, Khan *et al.* 2002). Due to its catabolic potential to degrade various LMW and HMW PAHs, the bacterium is considered a prototype for studying bacterial PAH degradation (Kim *et al.* 2010) and has been used for studies on microbiological soil decontamination *in vitro* (MacLeod and Daugulis 2003).

The degradation of B[*a*]P *in vitro* through *M. vanbaalenii* PYR-1 has resulted to date in the identification of *cis*-4,5-dihydro-4,5-dihydroxybenzo[*a*]pyrene, *cis*-11,12-dihydroxybenzo[*a*]pyrene, *trans*-11,12-dihydro-11,12-dihydroxybenzo[*a*]pyrene, 10-oxabenzo[*def*]chrysen-9-one, and various hydroxymethoxy- and dihydroxymethoxy derivates of B[*a*]P (Moody *et al.* 2004). Direct ring cleavage products were only detected as substrates when B[*a*]P was exchanged for B[*a*]P-4,5-diol. This resulted in the formation of the *ortho*-ring cleavage products 4-formylchrysene-5-carboxylic acid and 4,5-chrysene dicarboxylic acid (Moody *et al.* 2004). However, Schneider *et al*. were able to show for *Mycobacterium sp* RJGII-135 by means of mass spectrometry that the ring cleavage products 4,5-chrysene dicarboxylic

acid, dihydropyrene carboxylic acid and *cis*-4-(8-hydoxypyrene-7-yl)-2-oxobut-3-enoic acid are formed during growth on B[*a*]P (Schneider *et al.* 1996). Starting from the identified B[*a*]P metabolites, two different routes of degradation of B[*a*]P were posited for *Mycobacterium sp* RJGII-135.

In addition to the metabolites, various enzymes involved in PAH degradation have been identified and characterised (Kim *et al.* 2008). Specific ring oxygenases and quinone reductases have been identified. The first step is the hydroxylation of the PAHs through both monooxygenase and dioxygenase reactions, whereby 21 sequences for ring hydroxylating oxygenases (RHO) could be identified in the genome of *M. vanbaalenii* PYR-1 (Khan *et al.* 2001, Kim *et al.* 2006, Stingley *et al.* 2004).

In the 6.5 mb genome of *M. vanbaalenii* PYR-1, there is a 150 kb catabolic region between bases 494 – 643 kb. This region encodes enzymes for the degradation of PAHs. The genes are arranged in the form of gene clusters in atypical mosaic structures (Kim *et al.* 2008). This means a complex and irregular arrangement of genes for the required enzymes, as well as uneven distribution over different gene clusters. This and the variety of paralogues demonstrate high diversity, which is an important deciding factor in the overall number of PAHs that can be metabolised by *M. vanbaalenii* PYR-1 (Kim *et al.* 2008). To date, 67 proteins that are involved in PAH degradation have been identified. These include a large number of CYPs as well as dioxygenases, which are responsible for the initial oxidation of PAHs. Dehydrogenases, reductases, carboxylases and methylases have also been identified.

The initial oxidation of the aromatic system can take place through either dioxygenases or CYPs (Heitkamp *et al.* 1988). In addition to CYPs, epoxide hydrolases have been identified which are responsible for the formation of *trans*-dihydrodiols (Kweon *et al.* 2011). In contrast, only a small number of *cis*-dihydrodiol dehydrogenases and RHOs exist. Both the dihydrodiol dehydrogenases and the RHOs have a broad substrate spectrum and thus low specificity (Kweon *et al.* 2011). The cleavage of the aromatic ring of the catechol derivates can occur as either intradiol or extradiol cleavage (Kim *et al.* 2007, Kweon *et al.* 2007). The central product of the PAH degradation is protocatechuate, which is created during the degradation of various HMW PAHs and enters the tricarboxylic acid cycle as 3-oxoadipate after ring cleavage and decarboxylation (Kweon *et al.* 2011). In addition to typical metabolic enzymes, proteins for detoxification processes have also been identified. *O*-methyltransferases and *o*-quinone reductases neutralise the toxic aldehyde and hydroxyl quinones formed (Kim *et al.* 2005,

Vasiliou *et al.* 2000) and have a detoxifying effect through methylation or reduction (Kim *et al.* 2003, Kim *et al.* 2004).

Despite extensive research on *M. vanbaalenii* PYR-1, only a small number of metabolites originating from B[*a*]P have been identified to date. The ring cleavage products in particular, are largely unknown at this time and are merely posited based on the identified routes of degradation for naphthalene and phenanthrene. Proteomics data suggests that B[*a*]P is not attacked and cleaved at a specific ring, but that different routes of degradation to the initial ring cleavage exist that are initiated by both monooxygenases and dioxygenases (Kweon *et al.* 2011).

2. Objective

As products of combustion processes and components of carbon blacks and extender oils, PAHs and thus human exposure to PAHs are ubiquitous (statement of the BfR 025/2009, statement of the BfR 051/2009). Sources can be found not only in the environment, but also in many foods and everyday objects. This is an important reason for the in-depth investigation of the degradation and activation of PAHs in eukaryotes, mainly based on B[*a*]P as a widespread and procarcinogenic reference substance. In human cells, B[*a*]P is converted into BPDE by CYPs. BPDE is a strong electrophile that reacts with DNA and can therefore have a mutagenic effect (Dekant 2009, Luch 2005). Moreover, the field of environmental microbiology has shown that PAHs are widespread as xenobiotics and can be used by bacteria as a carbon and energy source. Microbial (aerobic) degradation frequently occurs here by means of oxygenated intermediates that have mostly not been identified to date and are often not further characterised in toxicological terms either (Juhasz and Naidu 2000, Peng *et al.* 2008). Against this background, the hypothesis has been put forward that the human microbiome contributes to the metabolisation of PAHs in the event of exposure and that the resulting metabolites could be carcinogenic.

The human skin is one of the organs with the highest exposure to PAHs. In addition, the skin microbiome is diverse and hosts a large number of species due to the many different microhabitats (Gao *et al.* 2007, Costello *et al.* 2009, Grice *et al.* 2009). Furthermore, some of the degraders of PAHs known from soil samples belong to genera of typical skin commensals, such as *Pseudomonas*, *Burkholderia*, *Rhodococcus* and *Mycobacterium* (Anderson *et al.* 1995, Marczynski *et al.* 2009, Peng *et al.* 2008, VanRooij *et al.* 1995). Due to the temperatures involved, bacterial growth and metabolisation on the skin are also slower than in the intestines so that a longer presence of excreted intermediates can be assumed (Chandler *et al.* 1975, Plank & Harvey 1979). In turn, this can lead to longer skin exposure to potentially carcinogenic metabolic products. In the case of PAHs, we can also assume changed resorption behaviour as well as possible skin sensitisation (Melikian *et al.* 1987, Anderson *et al*. 1995).

This thesis aims to clarify whether and how bacteria on human skin are able to metabolise PAHs and whether the metabolites formed in this process are of toxicological relevance to humans. The procarcinogen B[*a*]P is used as the lead substance because extensive reference data from eukaryotic metabolism is already available for this substance. There should be further

investigation into the extent to which bacterial PAH degradation is dependent on the test person and exposure, the role played by the relevant routes of degradation on the skin, and the metabolites that are formed and/or excreted during degradation. The identification of toxic intermediates which could potentially cause damage to the skin or skin cells is of paramount importance. In addition to their identification, the toxicological relevance of the intermediates, i.e. their effect and further activation/conversion through the human phase I metabolism, is relevant to research.

The topic of microbial PAH metabolism by the human microbiome has been examined for the first time in this thesis. We expect that this thesis as a whole will make an important contribution to clarifying the microbiome's role in the toxification of xenobiotics and thus help to better assess the risks posed by PAH exposure from consumer products such as cosmetics and plastics.

3. Results

3.1 Degradation of benzo[*a*]pyrene by bacterial isolates from human skin

Juliane Sowada, Achim Schmalenberger, Ingo Ebner, Andreas Luch and Tewes Tralau

Degradation of benzo[*a*]pyrene by bacterial isolates from human skin.

FEMS Microbiology and Ecology. 2014 Apr;88 (1):129-39. doi: 10.1111/1574-6941.12276.

online available: https://doi.org/10.1111/1574-6941.12276

The publication examines the ability of the human skin microbiome to metabolise polycyclic aromatic hydrocarbons. To this end, carcinogenic B[*a*]P was selected as the lead substance. Using 11 test participants, 21 bacterial isolates of human skin were acquired which were grown in a medium with B[*a*]P as the sole source of carbon and energy. Two different skin areas were sampled here: the forearm as a dry area and the neck as a moist skin area rich in sebaceous glands. With the help of 16S rRNA, 10 cultures from the forearm skin area and 11 isolates from the neck were identified. It was possible to allocate 11 of the 21 cultures to the *Micrococcus lutei* group. Additional cultures were identified as *Staphylococcus caprae, Staphylococcus sp., Bacillus licheniformis, Paracoccus yeei, Pseudomonas oleovorans, Acinetobacter iwoffii, Bacillus pumilus, Staphylococcus aureus* and *Pantoea agglomerans*. No specific connection could be established between age, sex or daily exposure to PAHs and the isolated pure cultures. With, doubling times of 13 hours to 3 days in minimal medium supplemented with 100 µM B[*a*]P the isolates grow comparatively slowly and show distinctly different growth curves. Supernatants of the media of 3-week-old bacterial cultures were analysed with a gas chromatograph with regard to their B[*a*]P content. After this period, the isolates were in the stationary growth phase, so that neither further growth was to be expected, nor high metabolic conversion rates. All ten forearm isolates showed B[*a*]P concentrations of 1.25 µM – 6.25 µM down from the originally supplied 100 µM B[*a*]P in the medium. The growth of the isolates with different PAHs as a source of carbon and energy was examined in a substrate spectrum with different LMW and HMW PAHs. The bacterial skin isolates were able to grow on other

PAHs, such as pyrene, fluoranthene, phthalic acid and catechol but – similar to B[*a*]P – with greatly differing doubling times. The disparate substrate spectrum, the different doubling times and the calculated molar mass balances indicate different B[*a*]P degradation routes. The mass balance relates to the bacterial biomass as protein formed per mole of carbon added. Whereas 6 g of protein per mol/ carbon equates to complete substrate metabolisation, a mass balance of 2.0 – 4.0 g protein per mol/ carbon equates to a partial utilisation of the substrate, as the supplied carbon source is not fully incorporated and used for the production of endogenous amino acids. Measured mass balances of 6.5, 4.9, 6.4 and 6.1 g protein per mol/ carbon for the cultures 3A, 3B, 1D and 2D indicate complete metabolisation of B[*a*]P, while there was only partial B[*a*]P degradation for all other cultures with measured mass balances of 1.3 to 2.9 g protein per mol/ carbon.

Molecular characterisation to identify the enzymes involved in degradation show that two genes were specifically activated under growth on B[*a*]P: a new flavin-dependent monooxygenase with sequence similarities to a DszaA/NtaA-like monooxygenase, and a NifH-like reductase. The monooxygenase was described during the degradation of simple xenobiotic terpenes such as limonene in *Rhodococcus jostii*. The NifH-like reductase in *Rhodopseudomonas palustris* CGA009 is involved in the degradation of the carbon skeleton of chloroalkanes. The genes were detected in the isolated bacterial pure cultures, as well as directly *in situ* in swabs of 2 cm^2 of skin.

The author‘s contribution:

Design of experimental approach (70%), conduction of experiments (90%), evaluation and interpretation of experimental data (70%), preparation of the manuscript (50%)

3.2 Toxification of polycyclic aromatic hydrocarbons by commensal bacteria from human skin

Juliane Sowada, Lisa Lemoine, Karsten Schön, Christoph Hutzler, Andreas Luch and Tewes Tralau

Toxification of polycyclic aromatic hydrocarbons by commensal bacteria from human skin

Archives of Toxicology. 2017 Apr 4. doi: 10.1007/s00204-017-1964-3.

online available: https://doi.org/10.1007/s00204-017-1964-3

The publication describes the toxicological relevance of prokaryotic B[*a*]P degradation of human skin isolates on the eukaryotic system. Out of 21 initially isolated bacterial strains 3 were characterised further with regard to metabolites formed and excreted during B[*a*]P degradation. The bacterial isolates *Bacillus licheniformis* 2C, *Micrococcus luteus* 1D and *Micrococcus luteus* 1B were grown in minimal medium with 100 µM B[*a*]P as the sole source of carbon and energy and the cultures sampled continually. This was followed by the examination of the cell-free culture supernatants on skin (HaCaT) and liver (HepG2) cells for their potential cyto- and genotoxicity. Bacterial supernatants of the early growth phase showed a cytotoxic effect on the sampled skin and liver cells. With the beginning of the exponential growth phase, cell-free supernatants of the partial B[*a*]P metaboliser *B. licheniformis* 2C continued to show toxicity which was also observed for bacterial supernatants of the stationary phase. Furthermore, the cytotoxic effect increased more strongly after treatment of HepG2 cells compared to HaCaT cells, which allows the conclusion of the further metabolisation of bacterial B[*a*]P metabolites by eukaryotic phase I metabolism. In addition to the cytotoxic effect of bacterial supernatants, it was also possible to show genotoxic effects of these supernatants. A total of 12 different metabolites were identified with the help of GC-MS analyses. B[*a*]P-1,6-dione, B[*a*]P-7,8-dione, B[*a*]P-6,12-dione, B[*a*]P-12-ol, B[*a*]A, B[*a*]A-11-ol and Phe-4-CO_2H were detected quantitatively, and B[*a*]P-1-ol, B[*a*]P-2-ol, B[*a*]P-4-ol, B[*a*]P-10-ol and B[*a*]A-1-ol qualitatively. A B[*a*]A-diol was also identified. The quantity as well as the composition of the metabolites varied between the strains tested, with Phe-4-CO_2H, B[*a*]A-11-ol, B[*a*]P-12-ol

and B[*a*]P-9,10-diol being only detected in *M. luteus* 1D. By contrast, B[*a*]P-6,12-dione was detectable exclusively in *M. luteus* 1B. With 4 – 277 nM, the concentrations of the identified metabolites were in the lower nanomolar range in the examined supernatants of all three isolates. Only the B[*a*]P-1,6-dione reached micromolar quantities, with 2.05 µM measured in medial supernatants of *M. luteus* 1D.

With the identification of a set of different bacterial B[*a*]P metabolites, it was to be investigated more closely whether the metabolites are responsible for the high cytotoxicity of bacterial supernatants. Cytotoxicity of single metabolites was observed exclusively for B[*a*]P-3-ol, B[*a*]P-7-ol, B[*a*]P-1,6-dione and B[*a*]P-7,8-dione. Mixtures of several metabolites identified in a specific bacterial strain and their concentration led to increased cytotoxicity. The synergistic effect could also be established when testing for genotoxicity via the COMET assay. Cells treated with mixtures of the individual metabolites showed increased DNA damage compared to cells treated with individual substances. All three extracts had a DNA-damaging effect in the COMET assay. This was also confirmed for *B. licheniformis* 2C and *M. luteus* 1B in the considerably less sensitive AMES test.

Cytotoxic bacterial supernatants induced the gene expression of *CYP1A1*, the typical enzyme of the eukaryotic phase I metabolism for activating the PAH metabolism in eukaryotic cells, in a strength similar to the one measured after treatment with 2 mM B[*a*]P. The metabolisation of B[*a*]P and B[*a*]P derivates takes place in eukaryotic cells via the cytochrome P450 monooxygenases CYP1A1 and CYP1B1, thus proving that *CYP* gene expression is induced by B[*a*]P and its bacterial metabolites.

The author‘s contribution:

Design of experimental approach (70%), conduction of experiments (90%), evaluation and interpretation of experimental data (70%), preparation of the manuscript (50%

3.3 The Q-rich/PST domain of the AHR regulates both ligand induced nuclear transport and nucleocytoplasmic shuttling

Anna Tkachenko, Frank Henkler, Joep Brinkmann, Juliane Sowada, Doris Genkinger, Christian Kern, Tewes Tralau & Andreas Luch

The Q-rich/PST domain of the AHR regulates both ligand induced nuclear transport and nucleocytoplasmic shuttling

Scientific Reports. 2016 Aug 18;6:32009. doi: 10.1038/srep32009.

online available: https://doi.org/10.1038/srep32009

The publication shows a new mechanism for the function of the AHR in the context of nucleocytoplasmatic shuttling and examines the underlying molecular mechanisms of AHR translocation. To examine the shuttling of the AHR between the cytoplasma and the nucleus, enhanced yellow fluorescent protein (EYFP) was fused to the N-terminus of the AHR. The fluorescence made it possible to observe the translocation of the AHR in real time in a confocal laser microscope, and to examine compartmentalisation.

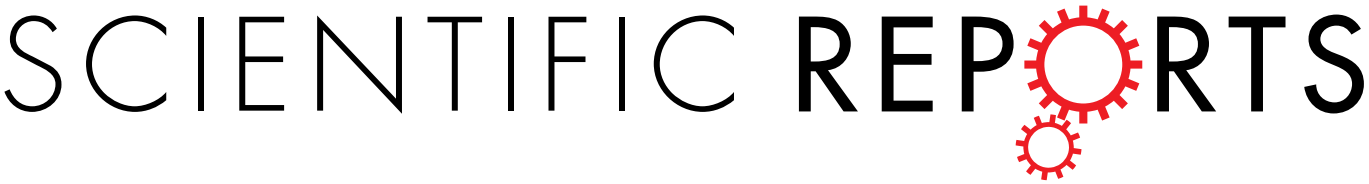
Even in the absence of ligands, a constitutive and highly dynamic shuttling of the AHR between the cytoplasma and the nucleus could be established in the examined HepG2 cells. Treatment of the transfixed liver cells with endogenous (Kyn), as well as exogenenous (β-NF) ligands led to an accumulation of the AHR in the nucleus, whereby ligand addition resulted in a doubling of the speed of the nuclear import compared to constitutive AHR shuttling. The addition of leptomycin B (LMB) resulted in an accumulation of the AHR in the nucleus, which inhibited the export of the receptor from the nucleus to the cytoplasma. Correspondingly *CYP1A1* and *CYP1B1,* two typical target genes of the AHR, were activated by the addition of 10 µM β-NF. Transcript levels for *CYP1A1* and *CYP1B1* accumulated 4-fold and 10-fold 40 and 60 minutes after treatment with β-NF, respectively. Induction of the *CYP* gene expression failed to occur after treatment of the HepG2 cells with LMB. The ligand-specific activation of the AHR import and export was confirmed in thermoshift assays with recombinant human AHR. The association

of recombinant AHR with Kyn and β-NF resulted in the stabilisation of the AHR-ligand complex in thermal stability measurements under real time conditions. A stabilising receptor-ligand complex was formed here in dependence of the ligand concentration, whereas LMB as an antagonist showed no stabilisation of the recombinant AHR of any kind in the tested concentration range.

In order to examine the regulation of the nucleus import as well as the export of the AHR, various deletion mutants were analysed more thoroughly. The long C-terminal deletions of the AHR mutants Δ509 and Δ391 showed an accumulation of the AHR in the nucleus, thereby confirming the basal constitutive import of the AHR as a key mechanism of the shuttling. Additional deletion mutants were analysed in order to examine the influence of other possible structural elements of the C-terminal domain of the AHR in more detail. These mutants of human AHR included deletions from amino acids Δ644 and Δ647. Whereas the mutant Δ644 accumulates in the nucleus, the extension by 3 amino acids to Δ647 causes a shift of the fluorescence signal in the direction of the cytoplasma. Closer examinations showed that the amino acid valine 647 plays a decisive role in the cytoplasmatic accumulation of the receptor. A deletion in the area of the glutamine-rich domain, which is also to be found in the C-terminal region of the AHR, from the amino acid Δ648 led to an exclusive cytoplasmatic localisation of the AHR. An acceleration of the core translocation could only be shown for these mutants through combined treatment with the ligands Kyn and LMB, as well as β-NF and LMB. This means that the glutamine-rich domain in connection with the amino acid valine 647 is important for the ligand-induced export inhibition of AHR from the nucleus. Moreover, a deletion in the region of the amino acids Δ648 – Δ661 results in the delay or failure of the translocation of the AHR in the nucleus after treatment with β-NF or Kyn. In addition to the regulation of the core export by the C-terminal domain, the PST domain, which is also localised at the C-terminus, was also identified as having an important influence on nucleocytoplasmatic distribution. Cytoplasmatic compartmentalisation is re-established with the extension of the deletion mutant up to amino acid Δ747. Addition of the ligands β-NF and Kyn confirms core translocation similar to the AHR wild type. Contrary to this, deletions from amino acid Δ723, as well as the NES domain, resulted in a predominant localisation of the AHR in the nucleus.

The author‘s contribution:

Design of experimental approach (10%), conduction of experiments (10%), evaluation and interpretation of experimental data (10%), preparation of the manuscript (10%)



OPEN

The Q-rich/PST domain of the AHR regulates both ligand-induced nuclear transport and nucleocytoplasmic shuttling

Received: 04 May 2016
Accepted: 01 August 2016
Published: 18 August 2016

Anna Tkachenko*, Frank Henkler*, Joep Brinkmann, Juliane Sowada, Doris Genkinger, Christian Kern, Tewes Tralau & Andreas Luch

The aryl hydrocarbon receptor (AHR) shuttles continuously between cytoplasm and nucleus, unless ligand-binding triggers association with the AHR nuclear translocator (ARNT) and subsequent binding to cognate DNA motifs. We have now identified Val 647 as mandatory residue for export from the nucleus and AHR-function. This residue prevents inactivation of the receptor as a consequence of nuclear sequestration *via* constitutive import. Concomitantly mutants lacking this residue are exclusively localised in the nucleus. Although ligands accelerate nuclear import transiently, stable nuclear transition depends on a motif adjacent to Val 647 that comprises residues 650–661. Together, this defined region within the Q-rich domain regulates intracellular trafficking of the AHR in context of both nucleocytoplasmic shuttling and receptor activation. Nuclear export therefore depends on the previously characterised N-terminal NES and the newly identified motif that includes V647. Nucleocytoplasmic distribution of full-length human AHR is further affected by a section of the PST domain that shows sequence similarities with nuclear export signals. In concert, these motifs maintain a predominant cytoplasmic compartmentalisation, receptive for ligand binding.

The AHR is a highly conserved protein belonging to the basic helix-loop helix (bHLH)-PAS family[1,2]. Originally identified for its association with xenobiotic ligands, particularly 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[3], this receptor became quickly recognised as one of the major regulators for eukaryotic phase-I metabolism. Further xenobiotic ligands include β-naphthoflavone (βNF), polychlorinated biphenyls, as well as carcinogenic polycyclic aromatic hydrocarbons (PAHs)[4]. Amongst other effects receptor activation induces expression of key enzymes of oxidative phase-I metabolism, notably cytochrome P450-dependent monooxygenases (CYPs) 1A1 and 1B1, both of which convert benzo[*a*]pyrene and other carcinogenic PAHs into highly mutagenic diol-epoxide intermediates[5]. Since then, certain endogenous ligands[6,7] and additional properties of the AHR have been widely explored. These studies revealed important physiological functions in the immune response[8], autoimmunity[9,10], carcinogenesis[11], apoptosis[12], skin differentiation[13], as well as in endocrine[14,15] and host-microbiome[16] signalling. Yet, comparatively little is known about its activation mechanisms that are tightly related with intracellular trafficking of receptor complexes. Notably, functional regulation might involve both nuclear import and export pathways. Prior to activation the AHR receptor is maintained in a cytoplasmic chaperone complex which consists of Hsp90, XAP2 and p23[17]. According to the current model, the ligand-bound AHR shifts to the nucleus and forms a heterodimer with ARNT to initiate transcription[18]. Importantly, constitutive nuclear translocation also occurs in the absence of ligand binding. This process is driven by ligand-independent import and balanced by parallel export in order to maintain a predominant cytoplasmic compartmentalisation. Although nucleocytoplasmic shuttling was recognised as early as 1998[19], both the physiological relevance and molecular mechanisms are merely understood.

Activation and intracellular trafficking of the AHR are regulated by the N-terminal domain that contains a bipartite nuclear localisation signal (NLS)[19], as well as an adjacent nuclear export signal (NES)[20] and the DNA-binding domain[21]. The well-characterised NLS is both required and sufficient to mediate import of

German Federal Institute for Risk Assessment (BfR), Department of Chemical and Product Safety, Max-Dohrn-Strasse 8-10, 10589 Berlin, Germany. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to A.L. (email: Andreas.Luch@bfr.bund.de)

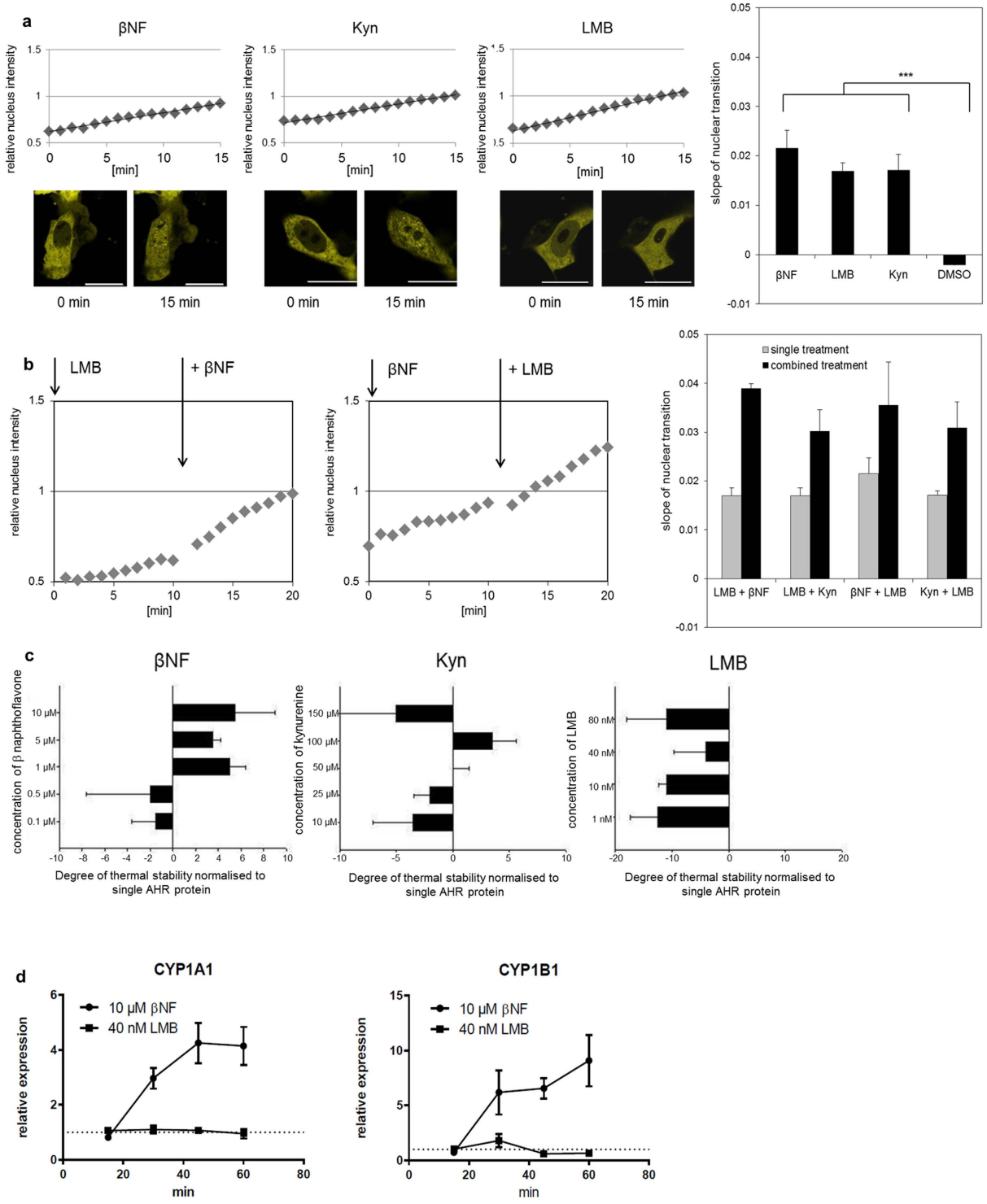


Figure 1. Constitutive nucleocytoplasmic shuttling of the AHR is accelerated by ligands. (**a**) Fluorescence images demonstrating the translocation of the full-length EYFP-AHR into the nucleus in HepG2 cells after treatment with 10 μM β-naphthoflavone (βNF), 100 μM kynurenine (Kyn) and 40 nM leptomycin B (LMB) for 15 min (scalebar = 20 μm). The selection of these concentrations was based on a dose-response analysis (Supplementary Fig. S2b). Graphs in the diagrams visualise the shift of nuclear staining in relation to total fluorescence of the analysed cells over 15 min. All three compounds triggered a comparable transition of EYFP-AHR into the nucleus, as indicated by comparable slopes of the graphs. Each bar (right diagram) represents the mean of at least 10 analysed cells +/− S.E.M. ***$p < 0.001$. (**b**) βNF accelerates the basal nucleocytoplasmic shuttling. Cells were treated for 10 min with 40 nM LMB, co-treated for another 10 min with 10 μM βNF (left side). Nuclear transition of EYFP-AHR was recorded and analysed as described above. Nucleocytoplasmic shuttling continues in the presence of ligand. Cells were treated with 10 μM βNF for 10 min, then co-treated with 40 nM LMB and analysed as described above (middle). Slopes of the recorded graphs were separately

determined for single and combined treatments as indicated (right side). Each bar represents the mean of at least 10 cells +/− S.E.M. (**c**) Thermal shift assay with purified recombinant human AHR (protein purification is summarised in Supplementary Fig. S4). Shown is the relative thermal stability of AHR in the presence of LMB, Kyn and βNF at different concentrations. Compared to unliganded AHR, βNF and Kyn increased thermal protein stability, while LMB apparently has a destabilising effect. A decrease of stability was noted using 150 μM Kyn, possibly triggered by precipitation. Shown are the means of three biological replicates +/− S.E.M. (**d**) Time-dependent transcriptional activation of CYP1A1 and CYP1B1 in HepG2 cells. Induction was only seen after treatment with 10 μM βNF but not with 40 nM LMB. Displayed values represent relative inductions of transcripts normalised to the solvent control. Values shown are means of three biological replicates +/− S.E.M.

AHR receptor complexes, leading either to receptor activation or to re-export into the cytoplasm, especially in the absence of ligands. Importantly, amino acid residues 63–73 comprise an export signal (NES)[19,20] which shows high sequence similarity to analogous viral and mammalian motifs[22]. This N-terminal NES overlaps with sequences that interact with the ARNT after ligand binding. It was hence postulated that nuclear export is blocked when this NES is masked by ARNT during receptor activation[23]. In consequence, AHR/ARNT complexes interact with xenobiotic response elements (XRE) to initiate expression of target genes[24], including CYPs 1A1 and 1B1[25]. Further, nuclear export of the AHR was discussed to terminate transcription and shown to trigger degradation *via* cytoplasmic proteasomes[23].

Several lines of evidence now suggest that nuclear export of the AHR depends on additional motifs or interacting factors. Notably, when the N-terminal NES was inactivated by mutagenesis, full-length AHR maintained a substantial cytoplasmic localisation in the absence of ligand[23]. In addition, several deletion mutants are exclusively localised in the nucleus[26] although the NES was not affected. It was speculated that these variant patterns are related to distinct interactions with XAP2, protein modifications or alternate conformations[26]. Moreover, the human receptor shifts from cytoplasmic staining towards a substantial nuclear staining pattern, when the C-terminal domain is replaced by its murine homolog[27]. Still, data on if and how the C-terminal domain contributes to nucleocytoplasmic shuttling and AHR compartmentalisation remain scarce. In this study, we have addressed the role of nucleocytoplasmic shuttling in relation to AHR activation and demonstrate an essential regulation of both receptor activation and intracellular trafficking by the C-terminal domain.

Results

Nucleocytoplasmic shuttling occurs as a constitutive and dynamic process. Previous investigations on the localisation of the AHR have mainly analysed cells that were fixed at defined time points and hence could only record cellular snap-shots. We therefore chose to investigate the cellular compartmentalisation of AHR in living HepG2 cells by live-cell fluorescence imaging using an expression construct that expresses the human matured protein (amino acids 11–848) fused to the C-terminus of enhanced yellow fluorescent protein (EYFP). The EYFP-tagged protein shows a shuttling behaviour similar to the wild-type protein, thus allowing continuous recording of functional AHR *in situ* (Fig. 1 and Supplementary Fig. S1). Our data confirm a constitutive and highly dynamic nucleocytoplasmic shuttling, since both, ligand binding of the xenobiotic βNF or endogenous kynurenine[6] (Kyn), as well as export inhibition by leptomycin B (LMB) resulted in a comparable accumulation of AHR in the nucleus (Fig. 1a,b). The latter compound was isolated from fungi and is known to affect CRM1-mediated nuclear export[19]. The binding of the ligands βNF and Kyn was confirmed by measuring AHR stabilisation in a thermal-protein shift assay, as was the absence of binding for LMB (Fig. 1c). The latter also failed to induce AHR-dependent target genes (Fig. 1d), further confirming its solely inhibitory function on nuclear export. The predominant cytoplasmic localisation of AHR is therefore maintained as a steady state by means of continuous parallel import and export, but shifts to nuclear accumulation after application of ligands. In addition the kinetic data show that while ligand binding accelerates the basal constitutive import, partial export continues to occur (Fig. 1b). As a consequence the ongoing export is likely to restrict the time frame for possible interactions of the respective receptor/ligand complexes with nuclear chromatin. It also indicates that corresponding molecular gene activation might actually require several passages of shuttling (Fig. 1b & see below).

Export of the AHR from the nucleus is strictly dependent on V647. Intracellular trafficking of the human AHR was further characterised using several deletion mutants. As previously observed for the murine protein by Pollenz and colleagues[26], extensive C-terminal deletion of murine AHR led to accumulation in the nucleus. In our experiments, an exclusive nuclear detection was observed for human $AHR^{\Delta 509}$ which lacks 339 amino acids at the C-terminus, as well as for the shorter variant $AHR^{\Delta 391}$ (Fig. 2). This observation confirms constitutive basal import of the AHR as key mechanism in shuttling. In contrast, the N-terminal NES acts not autonomously, but apparently requires either some additional factors or structural elements of the C-terminal domain. We explored the latter option by analysing several fluorescent mutants, namely $AHR^{\Delta 640}$, $AHR^{\Delta 644}$, $AHR^{\Delta 647}$, $AHR^{\Delta 650}$, $AHR^{\Delta 661}$ and $AHR^{\Delta 698}$. The data show a striking difference between $AHR^{\Delta 644}$ and $AHR^{\Delta 647}$. The inclusion of these three residues was sufficient to shift nuclear staining to a nearly exclusive cytoplasmic state (Figs 2 and 3). Further site directed mutagenesis of residues 645 to 647 (Fig. 2, see $AHR^{M645A,Q646A,V647A}$) led to an exclusive nuclear localisation of the full-length receptor. The respective locus seems therefore essential for any measurable export to happen. Further deletion mutagenesis confirmed V647 as critical residue (see $AHR^{\Delta 646}$, Figs 2 and 3).

We have applied additional site directed mutagenesis to confirm these findings (Fig. 2). While replacement of Q646 with alanine (AHR^{Q646A}) did not alter the cytoplasmic staining pattern of full-length AHR, replacement of V647 with alanine (AHR^{V647A}) did and led again to an exclusive nuclear staining. Interestingly, V647 is not conserved within in the AHR-sequence, but aligned to isoleucine in several species including mice. To test whether

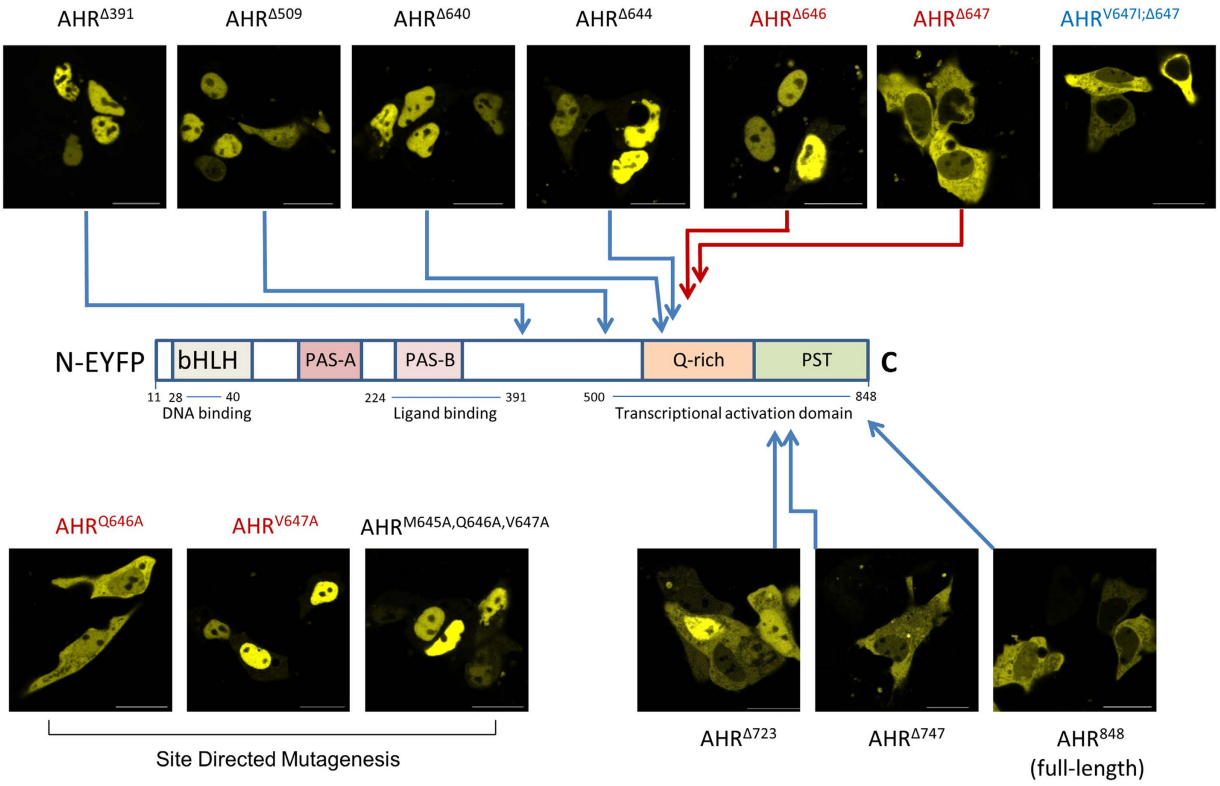


Figure 2. V647 determines the compartmentalisation of the AHR: expression of fluorescent AHR deletion mutants in HepG2 cells. Deletion mutants were derived from full-length human pEYFP-AHR-C1 (AHR^{848}). Mutants are named according to truncations sites, as defined by the last included residue. These sites are marked on the drafted full-length protein. Representative images that reflect the typical compartmentalisation are shown (scalebar = 20 μm). Mutants truncated after amino acid 391 ($AHR^{\Delta391}$), 509 ($AHR^{\Delta509}$), 640 ($AHR^{\Delta640}$) 644 ($AHR^{\Delta644}$) and 646 ($AHR^{\Delta646}$) show an exclusive nuclear staining, whereas the full-length protein (AHR^{848}) is predominantly located in the cytosol. $AHR^{\Delta647}$ is nearly exclusively detected in the cytoplasm. Inclusion of the Q-rich domain does increase nuclear association ($AHR^{\Delta723}$). This is balanced by a motif localised between Pro 728 and Leu 744 within in the PST domain. $AHR^{\Delta647}$ and full-length AHR^{848} show a similar predominantly cytoplasmic localisation. Replacing of residues M645, Q646 and V647 ($AHR^{M645A,Q646A,V647A}$), or V647 only (AHR^{V647A}) by alanines led to an exclusive nuclear staining, whereas mutant AHR^{Q646A} showed wild-type compartmentalisation (lower panel left). On the other side, replacement of V647 with isoleucine ($AHR^{V647I;\Delta647}$) did not affect the cytoplasmic staining pattern (upper panel right).

both amino acids are interchangeable, V647 was replaced with isoleucine in the $AHR^{\Delta647}$ mutant, thus creating $AHR^{V647I;\Delta647}$. In this experiment an exclusive cytoplasmic staining was confirmed similar to $AHR^{\Delta647}$. Therefore our data suggest equivalent properties of valine and isoleucine at residue 647 of the human AHR.

Activation-induced nuclear association of the AHR depends on the Q-rich domain. The C-terminal deletion mutant $AHR^{\Delta647}$ lacks essential parts of the Q-rich domain[28]. Notably this mutant showed an exclusive cytoplasmic staining that was markedly increased when compared with the wild-type receptor (Fig. 2). The deleted adjacent sequence is required for transcriptional activation of target genes *via* recruitment of co-activators and components of the basal transcription machinery[28]. Kinetic analysis of this mutant confirms its basal nuclear transition to be comparable to the full-length protein (Figs 1 and 4a). Similarly to the full-length receptor co-treatment with Kyn and the export inhibitor LMB accelerated the basal nuclear translocation, as did co-exposure to βNF and LMB. This was expected, since the deletion does neither affect the NLS, nor the ligand binding sites. Yet, contrastingly single substance exposure to Kyn or βNF failed to induce a stable or prolonged nuclear association of mutant $AHR^{\Delta647}$ (Fig. 4a). These data indicate that the Q-rich domain is required for ligand-induced export inhibition, likely involving further protein interactions. Consequently, interactions of ligand-bound AHR-complexes *via* the N-terminal domain are not sufficient to stabilise a nuclear fraction beyond the transient levels of shuttling. These findings propose the Q-rich domain as crucial switch to exit shuttling. Further analysis specified residues 648–661 as sufficient to maintain a stable nuclear fraction during activation (Fig. 4b,c). For these mutants, we have analysed the kinetics of nuclear transfer in the presence of agonists in detail. Our data demonstrate that the section between residues 648–661 is required and sufficient to facilitate nuclear accumulation of the AHR in response to ligands (Fig. 4c). Still, nuclear transition of the full-length receptor was slightly enhanced, pointing to the possibility that additional sites of the transactivation domain stabilise the nuclear fraction during ligand-induced activation.

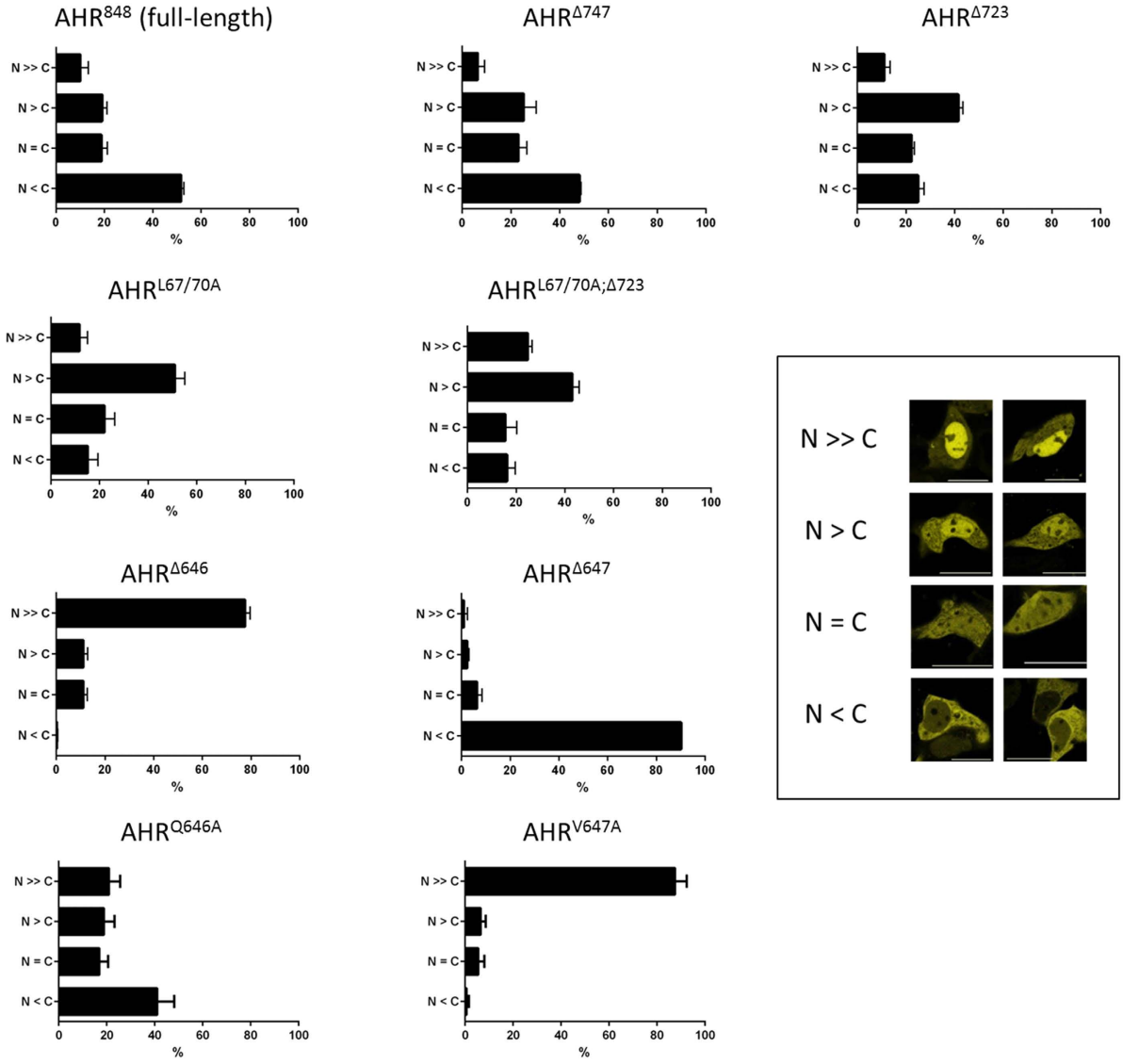


Figure 3. Compartmentalisation of EYFP-AHR mutants in transfected cells. A total of at least 300 positive cells that were found in randomly selected optical fields were analysed and classified after 24 h according to the defined staining patterns. Data represent the mean +/− S.D. out of three independent transfections. Insert: Staining patterns have been defined according to shown examples. N >> C exclusively nuclear; N > C predominantly nuclear; N = C equal distribution; N < C predominantly cytoplasmic.

The C-terminal PST domain affects the nucleocytoplasmic distribution of human full-length AHR. Microscopic analyses of $AHR^{\Delta 723}$ revealed a strongly enhanced nuclear staining in relation to $AHR^{\Delta 647}$ (Figs 2 and 3). Importantly, this staining was also distinguishable from the predominantly cytoplasmic pattern of the full-length AHR with its additional 125 amino acids. This finding raised the question on how the predominant cytoplasmic compartmentalisation is maintained. We analysed the C-terminal domain for relevant motifs, including potential export signals using LocNES predictor software[29]. The analysis yielded two high scoring hits that comprise two overlapping sequence motifs between Pro 728 and Leu 744 (Supplementary Fig. S3, PYPTTSSLEDFVTCLQL). Notably these motifs were not found in the partially homologous murine sequence, in which an alternate putative NES is predicted further upstream between Val 598 and Leu 612 by LocNES instead. Intriguingly, elongation of the human AHR protein to residue 747 ($AHR^{\Delta 747}$) restores the cytoplasmic compartmentalisation that is also seen with the wild-type receptor (AHR^{848}) (Fig. 2). Kinetic analysis of basal nucleocytoplasmic shuttling and ligand-induced nuclear translocation further confirmed the similarity to full-length AHR (Supplementary Fig. S2a). In contrast, the predominant nuclear compartmentalisation of $AHR^{\Delta 723}$ was further increased by concomitant inactivation of the N-terminal NES ($AHR^{L67/70A;\Delta 723}$). Deletion of both motifs doubled the proportion of cells that showed a nearly exclusive nuclear staining (Fig. 3). However, when this motif (i.e. Pro 728 – Leu 744) was fused to the C-terminal domain of EYFP, it did not shift the fusion protein towards an enhanced cytoplasmic distribution and no nuclear accumulation was observed after treatment with LMB

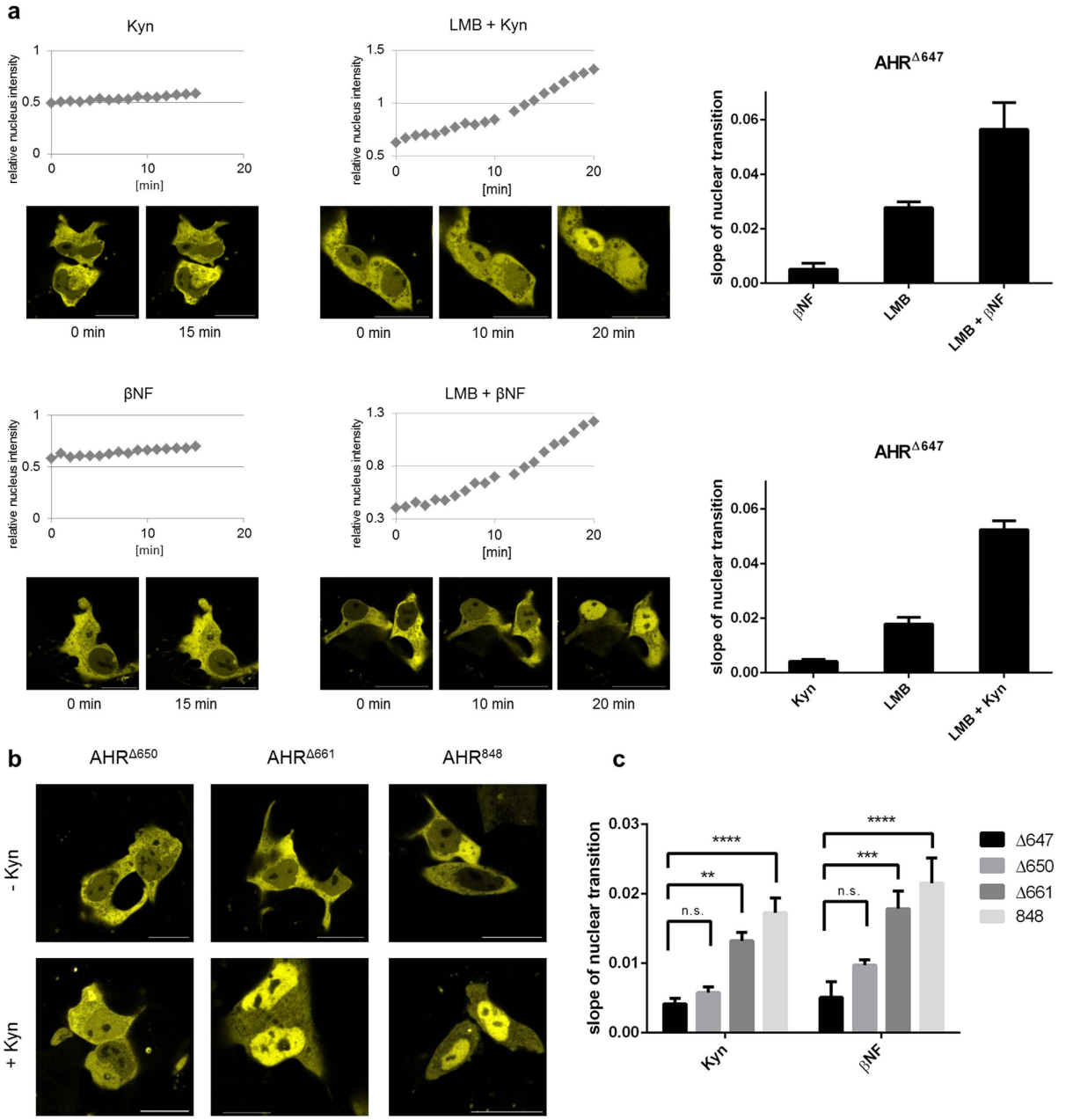


Figure 4. Ligand-induced nuclear association of the AHR depends on the Q-rich domain. (**a**) Translocation of EYFP-AHR$^{\Delta 647}$ into the nucleus in HepG2 cells after treatment with 100 μM Kyn or 10 μM βNF for 15 min (left). Translocation of AHR$^{\Delta 647}$ after exposure to 40 nM LMB for 10 min, followed by direct addition of 100 μM Kyn or 10 μM βNF for another 10 min (middle). Representative images of treated cells are shown for the indicated time points (scalebar = 20 μm). Nuclear transition was recorded and analysed as described in Fig. 1a. Slopes of the linear transition graphs have been separately determined for single and combined treatments (right). Each bar represents the mean +/− S.E.M. of 6 analysed cells. (**b**) Snapshots of cells imaged from transfected populations that were treated for 1 h with Kyn or left untreated. In response to ligand, AHR$^{\Delta 661}$ and full-length AHR^{848} showed a nearly exclusive nuclear staining pattern, while AHR$^{\Delta 650}$ remained predominantly cytoplasmic as in non-treated cells. Similar effects were observed after application of βNF. (**c**) Residues 648–661 are required for ligand-induced nuclear accumulation of the AHR. Cells expressing AHR$^{\Delta 647}$, AHR$^{\Delta 650}$, AHR$^{\Delta 661}$ or AHR^{848} (full-length) were treated with 100 μM Kyn or 10 μM βNF. Both, AHR$^{\Delta 661}$ and AHR^{848} showed significantly higher nuclear translocation rates than AHR$^{\Delta 647}$ (two-way ANOVA, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$). No such significant differences were observed between AHR$^{\Delta 647}$ and AHR$^{\Delta 650}$. Values depicted represent the mean +/− S.E.M of at least 5 cells.

(data not shown). Accordingly, a possible function as autonomous CRM1-dependent export signal could not be substantiated in this study. The observed effects on the cytoplasmic fraction therefore might rather depend on upstream elements, especially the N-terminal NES and the Q-rich domain.

Discussion

Functions of the AHR are closely related to intracellular trafficking both in the context of shuttling and ligand-induced nuclear transfer. According to the classical nuclear transport model, the AHR is complexed with Hsp90 and co-chaperones. Ligand binding had previously been proposed to stimulate release of Hsp90 from the N-terminal bHLH domain thus exposing the NLS and inducing transport of the AHR into the nucleus[30,19]. In addition, Richter and co-workers (2001) demonstrated that nuclear import can occur in the absence of xenobiotic ligands, but less efficiently[31]. Our data are in agreement with these previous observations and confirm a substantial acceleration of basal import by Kyn and βNF. Import of the AHR into the nucleus might also depend on endogenous ligands that could be formed in cultured cells as well. It seems conceivable that this process operates continuously, although less efficient in the absence of interacting ligands. According to the model proposed by Lees and Whitelaw (1999) interactions between Hsp90 and the bHLH are comparatively weak[30]. Therefore it might be well possible that the NLS is transiently exposed in a steady state proportion of receptor molecules that enter the nucleus.

The interplay between parallel import and export pathways provides a new perspective on the activation of the AHR shifting the focus from a sequential model to kinetic shuttling as a means of highly complex and fine-tuned receptor regulation. Firstly, the exclusive nuclear detection of deletion mutants supports the concept of a constitutively active NLS, which is also in agreement with rapid nuclear accumulation after export inhibition in LMB-treated cells[19,20,31]. Continual activation-independent export is therefore required to maintain a receptive cytosolic fraction of AHR complexes in waiting to respond to endogenous or xenobiotic (exogenous) ligands. Secondly, during activation, shuttling might assert an element of kinetic control not only balancing the molecule pools of AHR between different intracellular compartments (nucleus *vs.* cytoplasm), but also by crucially limiting the time frame for nuclear interactions of both the N-terminal and Q-rich domains in a ligand-dependent manner. We have summarised our conclusions in accordance to the analysed AHR mutants in Fig. 5. Importantly, the N-terminal NLS acts autonomously. Although it might be masked by interacting factors, no particular conformation or regulation by other AHR domains is apparently required for its functions. According to our data, this is a marked difference to the NES that is also localised in the N-terminal domain. The capacity of the N-terminal NES to shift AHR complexes out of the nucleus depends on a C-terminal section within the Q-rich domain that comprises V647 as mandatory residue. Possibly, this residue stabilises a conformation required for nuclear export, but apparently not for import. Further, the same region of the Q-rich domain is essential to consolidate a stable nuclear association during receptor activation. Again, this does not necessarily involve interactions with transcription factors or other proteins, but might be alternatively related to structural properties of the full-length protein.

Taken together, we have defined a section of the Q-rich domain that regulates export and trafficking of the AHR in the context of both nucleocytoplasmic shuttling and receptor activation. In addition, export regulation that involves the essential N-terminal NES is also affected by a further C-terminal motif within the PST domain.

Methods

Reagents and plasmids. Dimethyl sulfoxide (DMSO), β-naphthoflavone (βNF), leptomycin B (LMB), and kynurenine (Kyn) were obtained from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Munich, Germany). Full-length or truncated cDNA of human AHR were subcloned into pEYFP-C1 (Clontech), using the Bgl*II* and Kpn*I* sites. A unique forward primer 5′-catgacagatctgccagtcgcaagcggcggaag-3′ was used. Constructs encode the processed human AHR starting at Ala 11.

As reverse primers the following oligonucleotides were used. Full-length AHR^{848} (11–848): 5′-catgacggtaccttacaggaatccactggatgtcaaatc-3′; Truncated AHR variants:

$AHR^{\Delta391}$: 5′-acacaggtaccttatccttcctcatctgttagtgg-3′;
$AHR^{\Delta509}$: 5′-acacaggtaccttacaggatagtatcatttcccatc-3′;
$AHR^{\Delta640}$: 5′-acacaggtaccttactgacacagctgttgctgtgg-3′;
$AHR^{\Delta644}$: 5′-acacaggtaccagatctttagtgcttcatcttctgacacagctg-3′;
$AHR^{\Delta646}$: 5′- acacaggtaccgaattcttattgcatgtgcttcatcttctgacac-3′;
$AHR^{\Delta647}$: 5′-acacaggtaccaagcttaaacttgcatgtgcttcatcttctgacac-3′;
$AHR^{\Delta650}$: 5-acaca ggtaccttacatgccattaacttgcatgtgc-3
$AHR^{\Delta661}$: 5-acacaggtaccagatcttaaggcacgaattggttagagttcc-3
$AHR^{\Delta698}$: 5′-acacaggtaccttactgtgtataaggcatagaatcc-3′
$AHR^{\Delta723}$: 5′-acacaagcttggtaccttaactccccatagggtagtccagctc-3′;
$AHR^{\Delta747}$: 5′-acacaggtaccttagttttcaggaagttgtaaacaagtg-3′;
$AHR^{\Delta808}$: 5′-acacaggtaccttaatttaaaactccattctgaaacttg-3′.
$AHR^{V647I;\Delta647}$: 5′-acacaggtaccaagcttaaatttgcatgtgcttcatcttctgacac-3′;
pEYFPAHR$^{\Delta NES}$ variants that carry mutations at certain amino acids ($AHR^{L67/70A}$, $AHR^{M645A,Q646A,V647A}$, AHR^{Q646A}, AHR^{V647A}) were generated and sequenced by MGW Eurofins (Ebersberg, Germany).

Tissue culture and treatments. The human hepatoma cell line HepG2 was cultured in RPMI. Human embryonic kidney 293 (HEK293) cells were cultured in DMEM. Both cell lines were maintained in 5% CO_2 at 37 °C in culture medium containing 10% fetal calf serum (v/v), L-glutamine (2 mM), and penicillin/streptomycin (100 U/ml). All media components were purchased from Pan-Biotech (Aidenbach, Germany). For stimulation of cells, the media were replaced with fresh media or HBSS (Gibco - Thermo Fisher, Waltham, MA, USA) containing

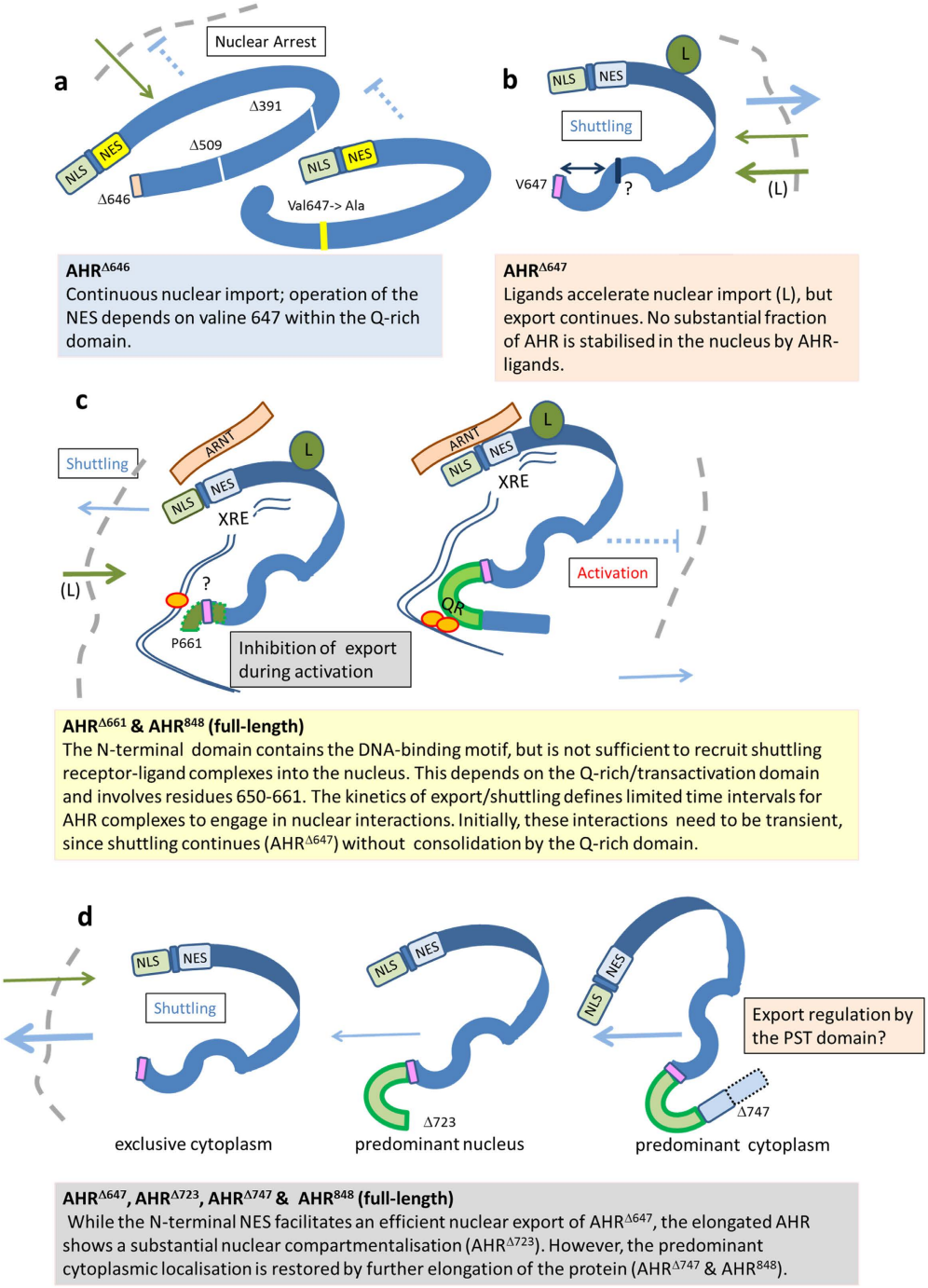


Figure 5. Nuclear export and intracellular trafficking of the human AHR are regulated by defined motifs. (**a**) The nuclear localisation signal (NLS) within the N-terminal domain triggers continuous basal import (green arrows) into the nucleus (shuttling). Contrary to this autonomous import mechanism, function of the adjacent nuclear export signal (NES) depends on C-terminal motifs, especially the mandatory residue V647. (**b**) Ligands (marked with L) accelerate import, while continued export (blue arrow) counteracts nuclear sequestration of the AHR, thus maintaining a predominant cytoplasmic fraction that is receptive for interactions with ligands. Notably, mutants that lack parts of the C-terminal domain (AHR$^{\Delta 647}$ and AHR$^{\Delta 650}$) do not efficiently accumulate in the nucleus, although nuclear transfer is accelerated by ligands. (**c**) Export of the AHR continues in the presence of ligands. Activation of the AHR might involve several passages of receptor molecules that need to engage in further associations with nuclear components during limited time intervals. Stable associations of the AHR with the nucleus likely require a defined section of the Q-rich domain (green, Pro 661 is indicated). However, it is as yet completely unknown how this motif stabilises nuclear compartmentalisation or whether it promotes interactions of the transactivation domain with transcription factors. (**d**) The N-terminal NES and the V647 motif facilitate an efficient nuclear export of AHR$^{\Delta 647}$, leading to a nearly exclusive cytoplasmic pattern. On the other side, the full-length AHR contains an additional motif within the PST domain to maintain a predominantly cytoplasmic compartmentalisation.

10 μM βNF, 100 μM Kyn or 40 nM LMB dissolved in DMSO/ethanol and further incubated for the indicated time period. Control cells were treated with solvent vehicle (0.1% DMSO) only.

Transient transfection. Depending on the further application, HepG2 cells and HEK293 cells were seeded either on 6-well plates (Techno Plastic Products AG, Trasadingen, Switzerland), glass-bottom dishes (*In Vitro* Scientific, Sunyvale, CA, USA), or on cover glasses (ThermoFisher Scientific, Loughborough, UK). At next day they were transfected with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) and an appropriate DNA concentration according to the manufacturer's instructions. For HEK293 cells, the surfaces were coated with poly-L-lysine (Biochrom AG, Berlin, Germany).

RNA analysis. RNA was isolated using RNeasy Mini kit in connection with the QIAshredder (QIAGEN GmbH, Hilden, Germany). The purity and the concentration of each RNA sample were determined using a NanoDrop1000 device (Peqlab Biotechnologie GmbH, Erlangen, Germany). The isolated RNA was used for cDNA synthesis with the High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR was carried out using an SYBR green assay (5Prime, Hamburg, Germany). Hypoxanthine guanine phosphoribosyl transferase (HPRT) was used as reference gene.

Western-blot analysis. Cells were lysed on ice in RIPA buffer containing 50 mM Tris/HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Igepal® and 0.25% sodium deoxycholate, with protease inhibitor cocktail (Calbiochem, San Diego, CA, USA). Equal amounts of proteins were applied to SDS-PAGE, transferred onto nitrocellulose membranes and immunoblotted according to the manufacturer's instructions. The primary antibody against AHR was used at 1:200 (sc-5579; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Primary antibody probed blots were visualised with appropriate horseradish peroxidase-coupled secondary antibodies (Santa Cruz Biotechnology) using enhanced chemiluminescence (34078; Thermo Scientific, Waltham, MA, USA) for detection.

Immunofluorescence staining. For immunofluorescence staining the Inside Stain Kit (Miltenyi Biotech Ltd., Bisley, Surrey, UK; Miltenyi Biotech Inc., Auburn, CA, USA) was used with minor modifications. After the fixation step, the cover glasses were incubated with the anti-AHR primary antibody (1:100, Santa Cruz Biotechnology) overnight at 4 °C. The slides were then washed and incubated with the secondary antibody for 30 min at room temperature (1:500, anti-rabbit FITC, Bethyl Laboratories, Inc., Montgomery, TX, USA). The slides were mounted in the VECTASHIELD HardSet Mounting Medium with DAPI (VECTOR LABORATORIES, INC., Burlingame, CA, USA), and then analysed using a confocal microscope (see below).

On-line confocal microscopy. For live-cell fluorescence imaging microscopy, HepG2 cells were seeded on glass-bottom dishes and transfected with plasmid DNA. Twenty four hours post transfection, fresh medium was applied and cells were monitored by confocal microscope. For on-line investigations, representative cells or cell groups were selected and maintained in buffered medium at 37 °C. Concurrently with treatments, live-cell imaging was started at a rate of one picture per minute. Typically, single treatment experiments were finalised after 15 min and combined treatment experiments after 20 min. Microscopic analyses and image acquisitions were done on an LSM 700 confocal microscope (Carl Zeiss Jena GmbH, Jena, Germany), using ZEN 2012 blue edition and ZEN 2011 black edition software (Carl Zeiss Jena GmbH). Data were analysed and graphed using Microsoft Excel and Prism Software (Graph Pad, La Jolla, CA, USA). Statistical analysis was done using two-way ANOVA and either Dunnett's or Sidak's multiple comparisons test. $\alpha = 0.05$; $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Cloning and expression of AHR. The human AHR DNA sequence was cloned into the pTFCold vector system (Clonetech, Takara) using a synthesised and codon-optimised construct. Clone identity was confirmed by sequencing and the respective plasmids where subsequently used for protein expression in the Rosetta 2 (DE3) system (Novagen) using 2xYT-medium and standard conditions with 300 μM isopropyl β-D-1-thiogalactopyranoside (IPTG), as recommended by the manufacturer. Following overnight cold-expression cells were harvested, washed (20 mM PIPES, 300 mM NaCl, pH 7.8) and subjected to lysis by sonication. At least 70% of expressed AHR were recovered in the soluble fraction and purified using Talon superflow agarose (GE Healthcare, Freiburg, Germany) according to manufacturer's instructions. After elution the protein concentration was estimated by spectrophotometric analysis and the purification efficiency was checked using SDS-PAGE (refer to Supplementary Fig. S4 for an exemplary gel).

Thermal shift assay. Thermal stability of AHR without and with ligands was assessed fluorometrically as described[32]. Assays were performed in a 96-well format using SYPRO orange in conjunction with the melting curve feature of a real-time PCR cycler. Following background subtraction the melting point was calculated based on the maxima of the melting curves using the equation of Biggar and co-workers[32].

Matrix-assisted laser desorption/ionisation Time-of-Flight (MALDI-ToF). We used MALDI-ToF to verify the identity of the recombinant human AHR protein. Following electrophoretic separation gel slices containing recombinantly expressed and purified AHR (~150 kDa) were cut out, destained, trypsin digested and subjected to fragment-pattern analysis using the following protocol. Destained gel slices were first reduced for 15 min at 60 °C using 100 mM ammonium carbonate with 45 mM dithiothreitol (DTT), and then alkylated for

15 min using 100 mM iodoacetamide. Following alkylation the gel slice was washed for three times and equilibrated for another 30 min in equilibration buffer containing 50 mM ammonium bicarbonate and 5% acetonitrile before commencing with overnight 4 ng/μL trypsin digestion at 37 °C. Following digestion the samples were extracted with 60% acetonitrile/0.1% trifluoroacetic acid (TFA) and 100% acetonitrile. Subsequently, samples were purified and desalted using C18 ZipTips (Merck Millipore, Darmstadt, Germany) with TFA supplemented (0.1%, v/v) washing solution. Eluted samples were spotted with α-cyano-4-hydroxycinnamic acid matrix on AnchorChip targets (Bruker, Rheinstetten, Germany) and analysed using an UltrafleXtreme MALDI-ToF/ToF (Bruker, Rheinstetten, Germany). Data evaluation was performed with ProteinScape (MASCOT/Swissprot database). The following search parameters were used: 1 missed cleavage, carbamidomethyl (Cys) as fixed modification and oxidation (Met) as variable modifications. Taxonomy was set to *Homo sapiens*. MS tolerance and MS/MS tolerance were set to 50 ppm and to 0.7 Da, respectively.

References

1. Gu, Y. Z., Hogenesch, J. B. & Bradfield, C. A. The PAS superfamily: sensors of environmental and developmental signals. *Annu. Rev. Pharmacol. Toxicol. A* **40,** 519–561 (2000).
2. Bock, K. W. & Kohle, C. The mammalian aryl hydrocarbon (Ah) receptor: from mediator of dioxin toxicity toward physiological functions in skin and liver. *Biol. Chem.* **390,** 1225–1235 (2009).
3. Poland, A., Clover, E., Kende, A. S., DeCamp, M. & Giandomenico, C. M. 3,4,3′,4′-Tetrachloro azoxybenzene and azobenzene: potent inducers of aryl hydrocarbon hydroxylase. *Science* **194,** 627–630 (1976).
4. Henkler, F., Stolpmann, K. & Luch, A. Exposure to polycyclic aromatic hydrocarbons: bulky DNA adducts and cellular responses. *EXS* **101,** 107–131 (2012).
5. Luch, A. Nature and nurture — lessons from chemical carcinogenesis. *Nat. Rev. Cancer* **5,** 113–125 (2005).
6. Stone, T. W., Stoy, N. & Darlington, L. G. An expanding range of targets for kynurenine metabolites of tryptophan. *Trends Pharmacol. Sci.* **34,** 136–143 (2013).
7. Fritsche, E. *et al.* Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. *Proc. Natl. Acad. Sci. USA* **104,** 8851–8856 (2007).
8. Esser, C., Rannug, A. & Stockinger, B. The aryl hydrocarbon receptor in immunity. *Trends Immunol.* **30,** 447–454 (2009).
9. Bessede, A. *et al.* Aryl hydrocarbon receptor control of a disease tolerance defence pathway. *Nature* **511,** 184–190 (2014).
10. Veldhoen, M. *et al.* The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* **453,** 106–109, doi: 10.1038/nature06881 (2008).
11. Murray, I. A., Patterson, A. D. & Perdew, G. H. Aryl hydrocarbon receptor ligands in cancer: friend and foe. *Nat. Rev. Cancer* **14,** 801–814 (2014).
12. Stolpmann, K. *et al.* Activation of the aryl hydrocarbon receptor sensitises human keratinocytes for CD95L- and TRAIL-induced apoptosis. *Cell Death Dis.* **3,** e388 (2012).
13. Fernandez-Salguero, P. M., Ward, J. M., Sundberg, J. P. & Gonzalez, F. J. Lesions of aryl-hydrocarbon receptor-deficient mice. *Vet. Pathol.* **34,** 605–614 (1997).
14. Ohtake, F. *et al.* Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. *Nature* **423,** 545–550 (2003).
15. Tarnow, P., Tralau, T. & Luch, A. G protein-coupled receptor 30 ligand G-1 increases aryl hydrocarbon receptor signalling by inhibition of tubulin assembly and cell cycle arrest in human MCF-7 cells. *Arch. Toxicol.* **90**, 1939–1948 (2016).
16. Tralau, T., Sowada, J. & Luch, A. Insights on the human microbiome and its xenobiotic metabolism: what is known about its effects on human physiology? *Exp. Opin. Drug Metab. Toxicol.* **11,** 411–425 (2015).
17. Barouki, R., Aggerbeck, M., Aggerbeck, L. & Coumoul, X. The aryl hydrocarbon receptor system. *Drug Metabol. Drug Interact.* **27,** 3–8 (2012).
18. Reyes, H., Reisz-Porszasz, S. & Hankinson, O. Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science* **256,** 1193–1195 (1992).
19. Ikuta, T., Eguchi, H., Tachibana, T., Yoneda, Y. & Kawajiri, K. Nuclear localization and export signals of the human aryl hydrocarbon receptor. *J. Biol. Chem.* **273,** 2895–2904 (1998).
20. Ikuta, T. *et al.* Nucleocytoplasmic shuttling of the aryl hydrocarbon receptor. *J. Biochem.* **127,** 503–509 (2000).
21. Fukunaga, B. N., Probst, M. R., Reisz-Porszasz, S. & Hankinson, O. Identification of functional domains of the aryl hydrocarbon receptor. *J. Biol. Chem.* **270,** 29270–29278 (1995).
22. Wen, W., Meinkoth, J. L., Tsien, R. Y. & Taylor, S. S. Identification of a signal for rapid export of proteins from the nucleus. *Cell* **82,** 463–473 (1995).
23. Davarinos, N. A. & Pollenz, R. S. Aryl hydrocarbon receptor imported into the nucleus following ligand binding is rapidly degraded via the cytosplasmic proteasome following nuclear export. *J. Biol. Chem.* **274,** 28708–28715 (1999).
24. Jones, P. B., Galeazzi, D. R., Fisher, J. M. & Whitlock, J. P. Jr. Control of cytochrome P1-450 gene expression by dioxin. *Science* **227,** 1499–1502 (1985).
25. Beischlag, T. V., Luis Morales, J., Hollingshead, B. D. & Perdew, G. H. The aryl hydrocarbon receptor complex and the control of gene expression. *Crit. Rev. Eukaryot. Gene Expr.* **18,** 207–250 (2008).
26. Pollenz, R. S., Wilson, S. E. & Dougherty, E. J. Role of endogenous XAP2 protein on the localization and nucleocytoplasmic shuttling of the endogenous mouse Ahb-1 receptor in the presence and absence of ligand. *Mol. Pharmacol.* **70,** 1369–1379 (2006).
27. Ramadoss, P. & Perdew, G. H. The transactivation domain of the Ah receptor is a key determinant of cellular localization and ligand-independent nucleocytoplasmic shuttling properties. *Biochemistry* **44,** 11148–11159 (2005).
28. Kumar, M. B., Ramadoss, P., Reen, R. K., Vanden Heuvel, J. P. & Perdew, G. H. The Q-rich subdomain of the human Ah receptor transactivation domain is required for dioxin-mediated transcriptional activity. *J. Biol. Chem.* **276,** 42302–42310 (2001).
29. Xu, D. *et al.* LocNES: a computational tool for locating classical NESs in CRM1 cargo proteins. *Bioinformatics* **31,** 1357–1365 (2015).
30. Lees, M. J. & Whitelaw, M. L. Multiple roles of ligand in transforming the dioxin receptor to an active basic helix-loop-helix/PAS transcription factor complex with the nuclear protein Arnt. *Mol. Cell. Biol.* **19,** 5811–5822 (1999).
31. Richter, C. A., Tillitt, D. E. & Hannink, M. Regulation of subcellular localization of the aryl hydrocarbon receptor (AhR). *Arch. Biochem. Biophys.* **389,** 207–217 (2001).
32. Biggar, K. K., Dawson, N. J. & Storey, K. B. Real-time protein unfolding: a method for determining the kinetics of native protein denaturation using a quantitative real-time thermocycler. *Biotechniques* **53,** 231–238 (2012).

Acknowledgements

Intramural funding of the BfR is gratefully acknowledged.

Author Contributions

A.T. carried out most parts of the experimental work and contributed towards planning and data processing and analysis. F.H. and A.L. designed the experimental program and contributed to data processing and analysis. C.K., J.B. and D.G. generated plasmids, contributed to the confocal microscopy studies, including data analysis. J.S. and T.T. planed and performed thermal shift assays and contributed to data analysis. The manuscript was written by F.H., A.T., T.T. and A.L.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Tkachenko, A. *et al.* The Q-rich/PST domain of the AHR regulates both ligand-induced nuclear transport and nucleocytoplasmic shuttling. *Sci. Rep.* **6**, 32009; doi: 10.1038/srep32009 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

© The Author(s) 2016

Supplementary information to:

The Q-rich/PST domain of the AHR regulates both ligand-induced nuclear transport and nucleocytoplasmic shuttling

Anna Tkachenko[†], Frank Henkler[†], Joep Brinkmann, Juliane Sowada, Doris Genkinger, Christian Kern, Tewes Tralau, and Andreas Luch*

Affiliation: German Federal Institute for Risk Assessment (BfR), Department of Chemical and Product Safety, Max-Dohrn-Strasse 8-10, 10589 Berlin, Germany.

*Corresponding author. Email: Andreas.Luch@bfr.bund.de; phone: ++49-30-18412-4538.

[†]These authors contributed equally to this work.

Fig. S1

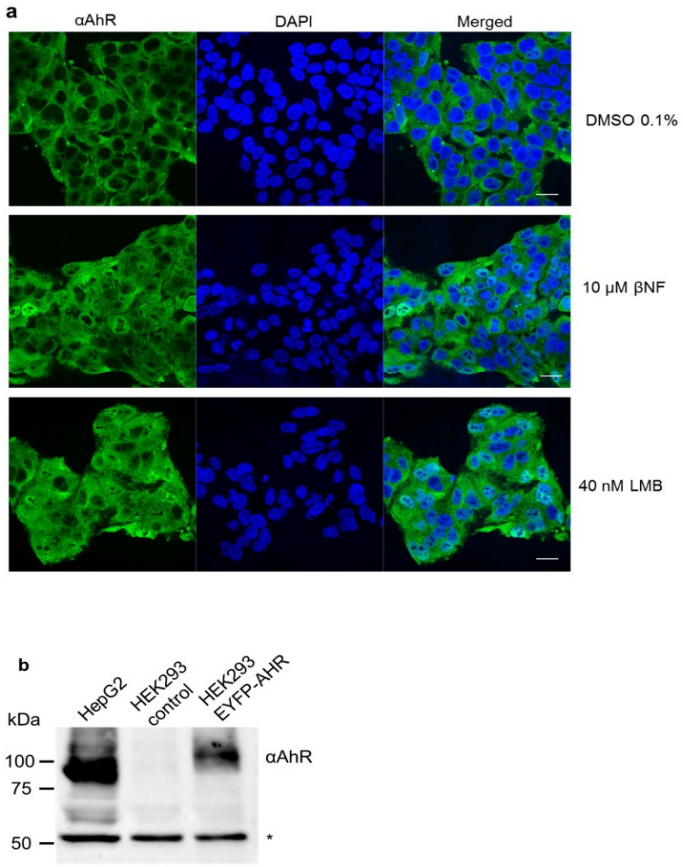


Figure S1. (**a**) Immunofluorescence staining of endogenous wild-type AHR in HepG2 cells after 1 h treatment with 10 µM βNF and 40 nM LMB (αAHR 1:100). Scalebar = 20 µm. βNF and LMB induce a similar nuclear translocation of the endogenous AHR. (**b**) Western-blot analysis of transfected human EYFP-AHR fusion protein in HEK293 cells, compared with endogenous AHR in HepG2 cells. *Cross-reaction bands indicate equal loading.

Fig. S2

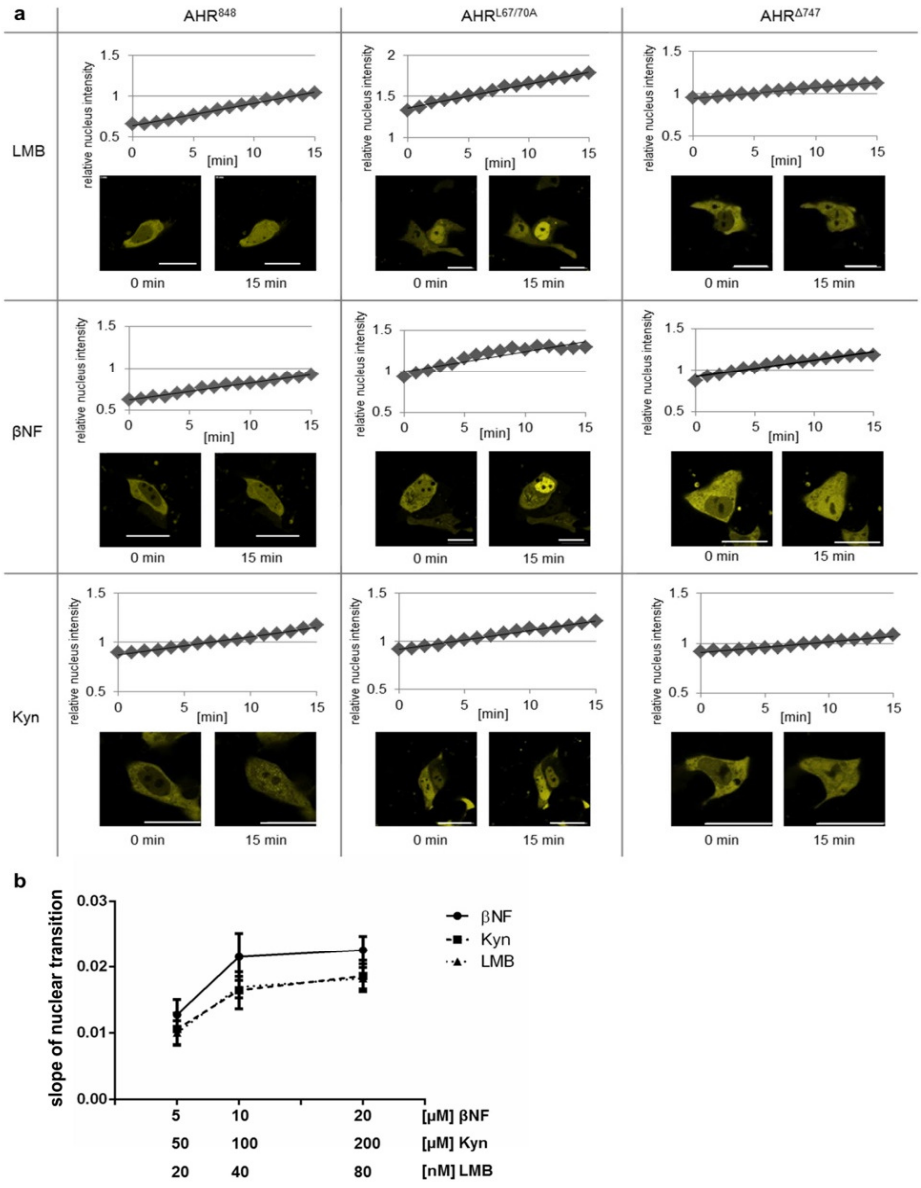


Figure S2. (**a**) Localisation and shuttling of full-length AHR (AHR^{848}), $AHR^{L67/70A}$ and $AHR^{\Delta747}$. Kinetics of protein translocation into the nucleus are shown during 15 min after treatment with 40 nM LMB, 10 µM βNF, or 100 µM Kyn. (**b**) Dose-dependent increases of the relative nucleus fluorescence intensity induced by βNF, LMB and Kyn. Cells treated with 10 µM βNF, 40 nM LMB and 100 µM Kyn show a similar nuclear fluorescence intensity increase, which significantly differs from solvent (DMSO) control cells (two-way ANOVA, p<0.0001=****). Values depicted represent the mean +/- S.E.M of 6 cells.

Fig. S3

Score human LocNES 510 – 748 (Gln 640 is labelled red):

KHEQIDQPQDVNSFAGGHPGLFQDSKNSDLYSIMKNLGIDFEDIRHMQNEKFFRNDFSGEVDFRDIDLTDEILTYV
QDSLSKSPFIPSDYQQQQSLALNSSCMVQEHLHLEQQQQHHQKQVVVEPQQQLCQKMKHMQVNGMFENWN
SNQFVPFNCPQQDPQQYNVFTDLHGISQEFPYKSEMDSMPYTQNFISCNQPVLPQHSKCTELDYPMGSFEPSPYP
TTSSLEDFVTCLQLPENQ

Protein Name	Position	Sequence	Score
>LocNES69844683_0	25-39	SKNSDLYSIMKNLGI	0.122
>LocNES69844683_0	30-44	LYSIMKNLGIDFEDI	0.152
>LocNES69844683_0	49-63	NEKFFRNDFSGEVDF	0.014
>LocNES69844683_0	54-68	RNDFSGEVDFRDIDL	0.026
>LocNES69844683_0	62-76	DFRDIDLTDEILTYV	0.030
>LocNES69844683_0	124-138	EPQQQLCQKMKHMQV	0.025
>LocNES69844683_0	219-233	PYPTTSSLEDFVTCL	0.338
>LocNES69844683_0	221-235	PTTSSLEDFVTCLQL	0.350

Figure S3. Analysis of the C-terminal domain of the human AHR protein, using the LocNES computational tool[29].

Fig. S4

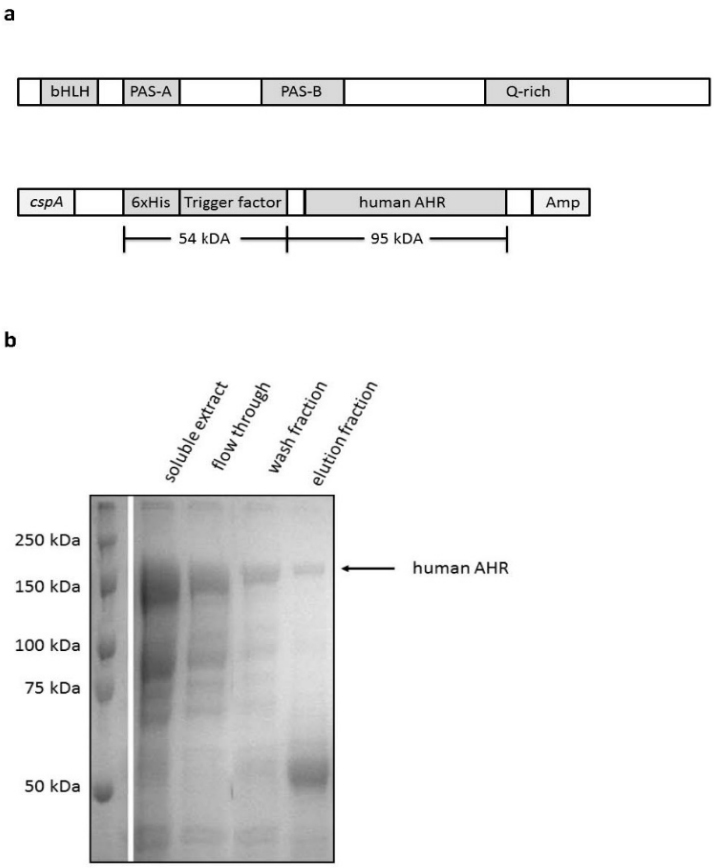


Figure S4. AHR expression construct for protein purification. (**a**) Domain structure of the human AHR. The bHLH, PAS and Q-rich domains are marked. A sketch of the generated AHR expression construct is given below. (**b**) Exemplary SDS-PAGE of AHR purification. The eluted fraction contains AHR protein and its degradation products as confirmed by MALDI-ToF (see section Methods).

4. Discussion

4.1 Bacterial B[*a*]P degradation on the skin

Polycyclic aromatic hydrocarbons have become ubiquitously distributed xenobiotics in the environment due to a high anthropogenic entry rate (Commins 1969, Morillo *et al.* 2007). Humans are exposed not only through particles in the air, soil and water but also through a large number of everyday consumer products such as beach sandals, the rubber coatings of tools and handlebars, and toys (statement of the BfR 025/2009, statement of the BfR 051/2009, statement of the BfR 032/2010). Moreover, the persistent HMW PAHs exhibit a carcinogenic effect after activation, or possess a procarcinogenic effect. The eukaryotic phase I and II processes for activating and detoxifying PAHs have been thoroughly examined. The model substance B[*a*]P can be converted via CYP P450 into BPDE, which has a highly carcinogenic and mutagenic effect due to the possibility of associating with human DNA (Dekant 2009, Luch 2009). Detoxification and excretion of substances through increased hydrophilicity can occur via conjugation processes, e.g. with glucuronides, mercapturic acid, acetate, methyl or sulphate groups, thiocyanates or glucosides (Shimada 2006). Although the prokaryotic metabolism of PAHs, especially B[*a*]P, has been less examined, processes which lead to the conversion and degradation of PAHs are mainly linked with the extraction of energy and carbon (Haritash and Kaushik 2009, Peng *et al.* 2008). Whereas the main selective pressure for a eukaryote is the maintenance of the entire organism, with bacteria it is mainly the supply of nutrients and thereby the exploitation of available sources. Aerobic degradation occurs via oxygenated interim products, most of which are unknown and have not been toxicologically examined in any detail either. The reactions are either catalysed via initial dioxygenases or, as in the eukaryotic metabolism, via cytochrome P450 enzymes (Peng *et al.* 2008).

For mammals, in addition to the lungs and intestines, the skin is strongly exposed to ubiquitous xenobiotics from the environment. Consequently, this results in exposure of the microbiome to xenobiotics such as PAHs. It is already known from the intestines that xenobiotic substances can be converted by the microbiome in the form of medications (Haiser and Turnbaugh 2013). In this way, frequently used active substances such as digitoxin, digoxin, phenacetin and sulfasalazine induce microbial genes which are associated with the transport and degradation of drugs (Maurice *et al.* 2013). In addition to an inactivation of the drug, as with digoxin through the bowel bacteria *Eggerthella lenta* (Haiser *et al.* 2013, Haiser *et al.* 2014, Lu *et al.* 2014), or

for example a desired activation of administered lovastatin, a lipid lowering drug, which is hydrolysed to the biologically active acid in the gastrointestinal tract (Yoo *et al.* 2014), cytotoxic effects can also result. Sorivudin is a chemotherapeutic agent which is used together with 5-fluorouracil as a tumour suppressor. By bowel bacteria, sorivudin can be converted into (E)-5-(2-bromovinyl)uracil which inhibits dihydropyrimidine dehydrogenase in the human organism, thereby resulting in a dangerous accumulation of 5-fluorouracil which can lead to lethal poisoning (Okuda *et al.* 1998, Ribeiro *et al.* 2016).

In addition to the conversion of drugs by the human intestinal microbiome, studies have demonstrated that the skin commensal *M. luteus* is capable of metabolising *in vitro* the azo dyes used in textiles (Platzek *et al.* 1999). This suggests that bacterial cultures of the human microbiome exhibit the potential of converting and degrading xenobiotic substances.

For this reason, the initial hypothesis of this thesis was that with the exposure to contaminants, bacteria exist on the human skin which can use xenobiotic substances as sources of carbon and energy.

4.1.1 Benzo[*a*]pyrene-degrading bacteria on the skin

The tests on samples under B[*a*]P selective pressure from eleven different test persons showed that, irrespective of age, sex, sampled skin area or PAH pre-exposure, 21 B[*a*]P-degrading bacterial isolates were identified on the human skin. This demonstrates that every test participant had commensals for which B[*a*]P represents a source of energy and carbon. Previous data on the human skin microbiome were based on analyses of the phylogenetic composition of the individual skin areas, as well as inter- and intraspecific diversity (Grice *et al.* 2008, Grice and Segre 2012). In this way, it was clarified which bacterial species settle on the skin. Moreover, the influence of abiotic and biotic factors on the microbial structures was examined more closely, such as natural ageing processes of the host, sex-specific differences, the extent of various diseases and exposure to contaminants and extreme weather conditions. Together with metabolomic analyses, these examinations provided a very good insight into the bacterial diversity and its metabolic competence. However, they do not reveal exactly which cultures are involved in certain metabolic and enzymatic processes. In this thesis, on the other hand, bacterial enrichment cultures were successfully cultivated *in vitro* for the first time. By doing so, more precise statements could be made on each respective metabolic process for the

metabolisation of B[*a*]P specific to a bacterial isolate, and these may then be examined in more detail.

The isolation of bacterial cultures from all test persons indicated that there has to be persistent exposure to PAHs such as B[*a*]P so that the metabolism on the skin is maintained and is not lost as a result of a lack of selective pressure. On the other hand, the consistent isolation of B[*a*]P-degrading bacterial cultures suggests that this is not an individual phenomenon. On the contrary, widespread inhabitation found on all test persons, also suggests permanent contact with contaminants such as B[*a*]P. According to the selective advantage, bacterial DNA sections/ operon structures would be lost for unused metabolic pathways via mechanisms such as natural competence or the genetic exchange of DNA between two bacterial cells. Two different skin areas were sampled: with the forearm a dry habitat and with the nape of the neck a moist, sebaceous skin region. Although very dry, the volar forearm is distinguished by high bacterial diversity (Gao *et al.* 2007, Grice *et al.* 2008), whereas the neck area has fewer species (Costello *et al.* 2009, Grice *et al.* 2009). The dominance of detected *Micrococci* isolates is conspicuous. The cocci which belong to the *Actinobacteria* makeup 40% of the cultures found on the forearm and 64% of the isolated neck cultures. They are typical representatives found on the skin which prefer a saprophytic mode of life. Studies have shown that *M. luteus* is only represented to 4% on average in the human skin microbiome (Costello *et al.* 2009, Ding and Schloss 2014, Gao *et al.* 2007, Grice *et al.* 2009). It is already known from environmental microbiology that *M. luteus* is involved in the degradation of contaminants, that it can tolerate toxic substances, and in particular that it is involved in the utilisation of xenobiotics as a source of carbon and energy (Ghosh *et al.* 2013, Haritash and Kaushik 2009, Peng *et al.* 2008). Confirmation that the identified isolates degrade B[*a*]P was provided by GC-MS analysis. The isolates grew in medium supplemented with 100 µM B[*a*]P for approx. three weeks. It was possible to detect a maximum of 10% of the utilised B[*a*]Ps in the medial supernatants, however. In this way, it was demonstrated for the first time that human skin isolates can metabolise B[*a*]P as the sole carbon and energy source and that this property appears to be ubiquitous. A total of 21 pure cultures from 11 different test persons were isolated. This suggests persistent and frequent exposure of the skin microbiome to higher molecular PAHs and is remarkable in that it has only been possible up to now to isolate two B[*a*]P-degrading pure cultures from soils (Gibson *et al.* 1975, Moody *et al.* 2004, Pinyakong *et al.* 2003).

4.1.2 Physiology and biochemistry of skin commensals and B[*a*]P degradation

The 21 bacterial isolates of human skin were subsequently examined with regard to their B[*a*]P degradation. Bacterial growth on B[*a*]P as the sole carbon and energy source was analysed initially. With doubling times of 13 hrs to 3 days, growth is comparatively slow. This is due on the one hand to an environmental temperature of about 30° C and can also be explained by the required time for substrate uptake and limited enzymatic activities on the other hand. Possible cytotoxicity or membrane toxicity of B[*a*]P on the bacteria can be excluded, as growth on LB with added B[*a*]P is not associated with distinctly longer growth of any isolate. The doubling times when grown exclusively on B[*a*]P differ greatly between the individual isolates. This can be an indication of different uptake and/ or transport pathways (Baboshin and Golovleva 2012, Moore and Harrison 1965). It is suspected for *M. vanbaalenii* PYR-1, for example, that due to the slimy, lipophilic surface, the B[*a*]P from the surrounding area can adhere better to the bacterium, thus potentially increasing the bioavailability of B[*a*]P. This causes an acceleration of B[*a*]P degradation (Haritash and Kaushik 2009). Moreover, the differing doubling times can be an indication of different B[*a*]P metabolic pathways. This would be consistent with the detected mass balances of the individual bacterial skin isolates. With approx. 6 g protein per mol/ carbon, the isolates 3A, 3B, 1D and 2D were complete B[*a*]P degraders. Contrary to this, the forearm isolates 2A, 1B, 2B, 1C and 2C metabolised B[*a*]P only partially as they have mass balances in the range of 2 g protein per mol/ carbon. This was further supported by differential growth on various other high and low molecular weight PAHs, such as phenanthrene, anthracene, pyrene and benzo[*a*]anthracene. Pyrene was used by a majority of the forearm isolates (2A, 1B, 2B and 1C) as an alternative carbon source, thus constituting a possible interim product of B[*a*]P degradation. A similar process has already been described by Kweon *et al.* (2011), who identified pyrene as an interim product of B[*a*]P degradation while examining *M. vanbaalenii* PYR1 with regard to the degradation of PAHs. With known three- and two-ring systems as additional interim products in the degradation of PAHs through soil bacteria (Heitkamp *et al.* 1988, Kim *et al.* 2005, Kweon *et al.* 2011, Moody *et al.* 2005, Peng *et al.* 2008), it is suspected that the skin isolates can metabolise LMW PAHs, but the growth on anthracene, phenanthrene or naphthalene does not occur with any of the isolates. On the one hand, this might be because the transport mechanisms required for uptake are missing, or on the other hand that metabolisation is not possible due to a lack of enzymes or activation.

To get an indication of the enzymes involved in B[*a*]P degradation, genes already known from soil samples as pollutant-degrading enzymes were used to identify relevant genes or gene sections with the help of degenerated primers. This included a flavin-dependent monooxygenase not previously characterised in any detail, with sequence similarities to a DszaA/NtaA-like monooxygenase and a NifH-like reductase. The expression of both genes was substrate-specific only with growth on B[*a*]P. Both genes were also detectable *in situ* in skin swabs from the test persons. Whereas the reductase was qualitatively detectable, the monooxygenase was present with up to 20,000 copies per cm^2 skin. In relation to the average inhabitation density of $10^2 – 10^3$ bacteria per cm^2, this is a comparatively large number of copies for a gene and speaks for the importance of the enzyme in the bacterial metabolism. The DszaA/NtaA-like monooxygenase has already been identified in the actinobacterium *Rhodococcus jostii*. This GC-rich bacterium was isolated on PAH-contaminated soils and is capable of degrading polychlorinated biphenyls and cyclic terpenes (Meynet *et al.* 2012). The monooxygenase plays an important role in the degradation of xenobiotic cyclic terpenes. In *R. jostii,* the monooxygenase is followed by an oxygenase which is involved in the degradation of cyclic terpenes such as limonene. It has to be assumed that although the enzyme plays an important role in the B[*a*]P metabolism, it does not occur in the initial stages of the activation of B[*a*]P because cyclic terpenes are not strongly hydrophobic and complex aromatic ring structures as the polycyclic aromatic five-ring B[*a*]P. Furthermore, dioxygenases in particular were identified as initial ring cleavage oxygenases or bacterial CYPs, mostly in mycobacteria. Kweon *et al.* (2011) also established that a significantly larger number of dioxygenases and monooxygenases are upregulated in the degradation of HMW PAHs than in the growth of the mycobacterium on LMW PAHs. Therefore, greater specificity of individual dioxygenases in PAH degradation was postulated with regard to high molecular and low molecular compounds. It should be mentioned that although the gene of the monooxygenase was activated specifically by B[*a*]P in the two *M. lutei* cultures, the enzyme could have other functions on the skin and convey the degradation of other/additional carbon sources. As monooxygenases have a wide distribution in various metabolic pathways, they are distributed in the DNA of other bacterial species, too. Moreover, the degradation of B[*a*]P is not necessarily linked specifically to the *M. lutei* species and if there is a great abundance of contaminants, genes and/or operon structures for the metabolisation of frequently occurring substances can also be passed on interspecifically via DNA exchange processes. BLAST results for the monooxygenase show great sequence similarities to *Micrococcus luteus* SK58 (98%) and *Rhodococcus erythropolis* SK121 (79%),

the two typical bacterial representatives on the skin. Accordingly, the DszaA/NtaA-like monooxygenase can be assigned to the skin commensals. The NifH-like reductase has already been identified in *Rhodopseudomonas palustris* CGA009 as an enzyme involved in the degradation of chloroalkanes (Larimer *et al.* 2004). This bacterium occurs in marine environments and in the soil and has a very diverse metabolism which can alternate between a photoautotrophic, photoheterotrophic, chemoautotrophic and chemoheterotrophic modes of life (Pant *et al.* 2010). In addition, *Rhodopseudomonas palustris* CGA009 is a part of the consortia involved in the biological degradation of contaminated industrial sites (Oda *et al.* 2003). Due to the degradation of mainly aliphatic xenobiotic substances, the enzyme probably plays an active part in the downstream parts of B[*a*]P degradation. This is caused among other things by stereospecific backgrounds between very large HMW PAHs and very small short-chain aliphatic chloroalkanes.

4.2 Excretion of toxicologically relevant B[*a*]P metabolites on the skin

4.2.1 Identification of B[*a*]P metabolites

In order to examine in more detail the formation of metabolites during B[*a*]P degradation and their influence on the human host, three different forearm isolates were selected: *M. luteus* 1D, *M. luteus* 1B and *B. licheniformis* 2C. These isolates degrade B[*a*]P partially or fully and enable a comparison of the metabolic pathways of the two *M. lutei* isolates. In the bacterial supernatants which were removed daily from growing bacterial liquid cultures, it was possible to identify ten different metabolites that were excreted from the skin isolates into the medium. The B[*a*]P metabolites B[*a*]P-9,10-diol, B[*a*]P-1,6-dione, B[*a*]P-6,12-dione, B[*a*]P-7,8-dione, B[*a*]P-3-ol and B[*a*]P-7-ol are already known from the eukaryotic metabolism (Baird *et al.* 2005, Dekant 2009, Lin *et al.* 2016, Lorentzen and Ts'o 1977, Luch 2009, Shimada 2006, Souza *et al.* 2016, Sulc *et al.* 2016). The identified metabolites B[*a*]P-12-ol, B[*a*]A, B[*a*]A-11-ol and Phe-4-CO_2H are not known from the eukaryotic B[*a*]P metabolism and, to date, have not been described in the literature on the prokaryotic degradation of B[*a*]P either. In addition to the bacterial isolates identified in this paper, the use of B[*a*]P as the sole source of carbon and energy has been described for the model organism *M. vanbaalenii* PYR-1 (Moody *et al.* 2004). Only three of the bacterial B[*a*]P metabolites identified here were also identified in *M. vanbaalenii* PYR-1, namely B[*a*]P-1,6-dione, B[*a*]P-3-ol and B[*a*]P-7-ol. With the

identification of the four-ring (B[*a*]A and B[*a*]A-11-ol) and three-ring (Phe-4-CO_2H) systems, it was possible to identify ring cleavage products of B[*a*]P that prove the degradation of the aromatic five-ring system and show further degradation of B[*a*]P through *M. luteus* 1D with the three-ring system Phe-4-CO_2H. The two partial B[*a*]P degraders *B. licheniformis* 2C and *M. luteus* 1B only excreted B[*a*]P metabolites as well as the four-ring system B[*a*]A. No other low molecular weight compounds or oxidised four-ring systems were detected. The accumulation of remaining five-ring or four-ring metabolites in the bacterial medium might correspond to the identified partial degradation based on the measured mass balances (*M. luteus* 1B 2.9 g protein/ mol carbon; *B. licheniformis* 2C 1.6 g protein/ mol carbon). In contrast, with respect to the complete degrader *M. luteus* 1D, multiple four-ring and three-ring compounds could be identified with B[*a*]A, B[*a*]A-11-ol and Phe-4-CO_2H. These show the further degradation of B[*a*]P. The findings are supported by the calculated mass balance of 6.4 g protein/ mol carbon for complete degradation of B[*a*]P (Sowada *et al.* 2014). The result of different B[*a*]P degradation is also proven by the different substrate spectra for the three bacterial isolates examined (Sowada *et al.* 2014). In contrast to the eukaryotic metabolism, primary oxidation does not take place on the fourth ring (Luch 2009, Shimada 2006); but rather, initial oxygenation seems to take place on the fifth ring. After ring cleavage, this then inevitably results in the identified B[*a*]A. This is consistent with the detection of B[*a*]P-12-ol in supernatants of *M. luteus* 1D. The identification of Phe-4-CO_2H, which was found exclusively in supernatants of *M. luteus* 1D, the only complete degrader of the three examined skin isolates, confirms the result of further B[*a*]P degradation. In contrast, the two partial B[*a*]P metabolisers *B. licheniformis* 2C and *M. luteus* 1B only excrete four- and five-ring systems. With no further metabolism, the metabolites in question accumulate in the medium and thereby potentially on skin. Additional excreted intermediates from the bacterial B[*a*]P metabolism were present, but could not be categorised due to a lack of reference substances and libraries. In particular, similar PAHs such as B[*a*]A and chrysene cannot be differentiated from one another in the chromatogram without reference libraries or explicit detection of the precursors. Aside from the sensitivity of the method, the time window of the excretion is also one of the main problems. The excretion of intermediates of B[*a*]P degradation is time- and phase-dependent. Thus, for example, metabolites are excreted temporarily during accumulation and taken up again at a later time to be further metabolised. It is therefore difficult to make a prediction on the time of metabolite excretion. Furthermore, different metabolites are excreted transiently and incorporated again at different times, particularly for the complete degrader *M. luteus* 1D.

Another factor to be considered is that only very small amounts of individual intermediates are excreted by the skin isolates under certain circumstances, and such low amounts cannot be analysed with respect to their quantities in the medium. Metabolites that could be determined qualitatively but not quantitatively were B[*a*]P-1-ol, B[*a*]P-2-ol, B[*a*]P-4-ol, B[*a*]P-10-ol and B[*a*]A-1-ol. Moreover, using mass spectrometry, an additional B[*a*]A-diol was detected but has not been categorised to date because of a lack of reference substances. Similar to *M. vanbaalenii* PYR-1, the different hydroxylated B[*a*]P intermediates could indicate the use of different routes of degradation within a bacterium (Schneider *et al.* 1996).

4.2.2 Toxicological relevance of bacterial supernatants and identified B[*a*]P metabolites

The toxicological relevance of microbial B[*a*]P degradation on the skin was investigated by means of cytotoxic and genotoxic studies on skin cells. For this purpose, cell-free supernatants of *M. luteus* 1D, *M. luteus* 1B and *B. licheniformis* 2C were studied with respect to their cytotoxic effect using bacterial cultures sampled daily. Testing was performed on the extensively studied immortal skin cell line HaCaT (Boukamp *et al.* 1988, Schoop *et al.* 1999) and primary normal human epidermal keratinocytes (NHEK), as a surrogate for skin. The immortal liver cell line HepG2 was used as an additional cell line. Because of its increased basal CYP expression, it has significantly higher metabolic competence as compared to the skin cells (Oesch *et al.* 2014, Swanson 2004). Thereby, the liver cells enable the examination of further metabolisation in the eukaryotic phase I metabolism of secreted bacterial B[*a*]P metabolites. The toxicity of bacterial supernatants could be observed both in HaCaT cells and in NHEK and HepG2 cells, with a maximum cytotoxicity of 40% (HaCaT), 30% (NHEK) and 60% (HepG2). A comparison of the individual cells and cell lines shows that the cytotoxicity in HepG2 cells is significantly more pronounced than that in the HaCaT and NHEK cells. This effect may be explained by the previously mentioned higher metabolic competence of the liver cells, which is associated with a high basal level of expressed CYPs and has the result that bacterial metabolites are further converted in the phase I metabolism. The differential metabolic competence of HaCaT and HepG2 cells is reflected in the measured EC_{50} values for the procarcinogen B[*a*]P, which demonstrates no cytotoxicity as the original substance and only has a cytotoxic and carcinogenic effect after metabolic activation. HaCaT cells exhibit an EC_{50} value of 54 µM while HepG2 cells exhibit an EC_{50} value of 18 µM. However, both HaCaT and

NHEK show a marked reduction in cell viability after treatment with bacterial supernatants. Therefore, the secreted bacterial metabolites already have high cytotoxicity which is independent of further metabolic conversion in the eukaryotic Phase I metabolism. As compared to HepG2 cells, both HaCaT and NHEK cells exhibit a significantly lower metabolic potential.

Cytotoxic effects can be observed with the beginning of the early growth phase of the three bacterial isolates. Depending on the bacterial route of degradation of the B[*a*]P (Sowada *et al.* 2014), a transient (*M. luteus* 1D) or persistent (*B. licheniformis* 2C, *M. luteus* 1B) cytotoxic effect can be observed in HaCaT and HepG2 cells. The transient loss of viability is caused by the likewise transient excretion of B[*a*]P metabolites. If the metabolites are taken up by the isolates once again, no cytotoxic effect of the bacterial supernatants in the eukaryotic cells can be observed. In contrast, during the partial metabolisation of B[*a*]P, an accumulation of cytotoxic B[*a*]P metabolites takes place in the medium, so that a persistent cytotoxic effect in the eukaryotic cells occurs from a bacterial growth duration of approximately five days (*M. luteus* 1B) or nine days (*B. licheniformis* 2C). Both the persistent and the transient loss of viability of the cells after treatment with bacterial supernatants confirm the different dominant B[*a*]P routes of degradation of the individual human isolates by biochemical and physiological studies (substrate spectra and mass balances) (Sowada *et al.* 2014).

An increase in the *CYP1A1* gene expression by a factor of 100 after treatment of HaCaT cells with cell-free bacterial supernatants is of the same magnitude as the 122-fold *CYP1A1* gene induction measured with 2 mM B[*a*]P. Benzo[*a*]pyrene, as a procarcinogen, is a strong inductor of the *CYP1A1* and *CYP1B1* gene expression in human keratinocytes. Metabolic conversion can have detoxification as a consequence, but it can also lead to a further increase in the existing toxicity. The increase in the *CYP1A1* gene expression can be attributed to an induction by the bacterial metabolites, because analytical measurements have shown that a maximum of 5 µM B[*a*]P is contained in the bacterial supernatant in the late logarithmic phase (Sowada *et al.* 2014).

The measured cytotoxicity of the bacterial supernatants is of roughly the same magnitude as of known, highly carcinogenic PAHs such as dibenzo[*a,l*]pyrene (DB[*a,l*]P). The six-ring PAH DB[*a,l*]P is a carcinogen 30 to 100 times stronger than B[*a*]P (Siddens *et al.* 2012). This is reflected in the measured EC_{50} values both for B[*a*]P with 54 µM (HaCaT) and 18 µM (HepG2) and for DB[*a,l*]P with 0.8 µM (HaCaT) and 0.4 µM (HepG2). The measured concentrations of the identified bacterial metabolites are within the nanomolar range. Only B[*a*]P-1,6-dione has

a concentration of 2 µM. The cytotoxicity of the metabolites is thus in the same range as the highly carcinogenic DB[*a,l*]P. This is concerning, because eukaryotic metabolites such as BPDE and B[*a*]P-1,6-dione are already considered highly reactive and carcinogenic (Lloyd and Hanawalt 2000, Luch 2005, Luch 2009).

Moreover, all bacterial supernatants exhibit a DNA-damaging effect. The measured genotoxic potential of the bacterial supernatants is comparable with that of B[*a*]P, with a relative DNA damage of 40%. This means that excreted bacterial metabolites from three different human skin isolates have a considerable cytotoxic and genotoxic effect. The corresponding cell-free supernatants originate from the middle and late growth phase and thus from growth phases in which more than 95% of B[*a*]P has already been used up. The DNA damage measured can therefore be attributed to excreted metabolites. With a relative DNA damage to the HaCaT cells of 40 or 50%, both *M. luteus* cultures excrete metabolites with an increased genotoxic potential as compared to *B. licheniformis* 2C with relative DNA damage to the HaCaT cells of 27%.

To examine whether the measured cytotoxicity and genotoxicity result from the identified metabolites of the prokaryotic B[*a*]P degradation, the individual metabolites were used in their measured concentrations to treat HaCaT and HepG2 cells. Cytotoxic tests with the maximum concentration of the respective identified bacterial metabolite showed a weak cytotoxic effect as compared to the bacterial culture supernatants. B[*a*]P-7,8-dione and B[*a*]P-1,6-dione as well as B[*a*]P-3-ol and B[*a*]P-7-ol showed a loss in viability of approximately 10%. For B[*a*]P, B[*a*]P-7,8-dione and B[*a*]A, cytotoxicity is proven and is within the nanomolar or micromolar range of concentration (Behrens *et al.* 2001, Burczynski and Penning 2000, Creusot *et al.* 2015, Kim *et al.* 1998, Shimada 2006). None of the identified substances on their own cause a cytotoxic effect that is equivalent to the cytotoxicity of bacterial supernatants. This leads to the conclusion that not all toxicologically relevant metabolites were identified and that combinations of the individual substances have a synergistic effect on cytotoxicity (Steliga *et al.* 2012). It was possible to confirm the synergistic effect by means of the reconstruction of bacterial supernatants based on the maximum concentrations of the identified bacterial metabolites. A comparison of the cytotoxicity of the substance combinations with the toxicities of the bacterial supernatants showed a similar cytotoxic effect in HaCaT and HepG2 cells. The synergism of the metabolite mixtures could also be observed in the damage to the DNA of HaCaT cells. After treatment of the HaCaT cells with reconstituted metabolite mixtures, DNA damage of approximately 50% could be detected. This is equivalent to the genotoxicity of bacterial culture supernatants. The potential of DNA damage for the metabolites found was

only described for B[*a*]P-1,6-dione. Diones are quinones that constitute electrophiles which can interact very well with the purinated bases of the DNA and thus cause the damage (Baird *et al.* 2005, Burczynski and Penning 2000, Lorentzen and Ts'o 1977).

At present, only two bacteria that use B[*a*]P as the sole source of carbon and energy are known from the literature: *Mycobacterium vanbaalenii* PYR-1 and *Sphingomonas paucimobilis*. Furthermore, there is very little knowledge of the metabolites, particularly ring degradation products, of B[*a*]P. Until now, even less was known about how the toxicity of prokaryotic metabolites formed. The present data therefore reveals the toxicological relevance of bacterial B[*a*]P degradation on the skin for the first time. The identification of bacterial B[*a*]P metabolites showed that bacterial isolates of the human skin metabolise B[*a*]P and form 13 hydroxylated five-ring B[*a*]Ps, four-ring PAHs and three-ring PAHs in the process. The data acquired on cytotoxicity and genotoxicity indicates that not all metabolites were identified. However, a high cytotoxic effect could be detected *in vitro*, correlating with significant DNA damage in HaCaT cells and primary human keratinocytes. Due to the slow metabolism of the isolates on the skin with doubling times of 1 d – 3 d, the presence time of excreted metabolites is approximately four days. The metabolites of the two partial B[*a*]P metabolisers *B. licheniformis* 2C and *M. luteus* 1B accumulate outside the bacterial cell. If penetration of the skin occurs, this can result in damage to the skin cells, which could ultimately lead to carcinogenesis. It has already been shown on pig skin that different PAHs, including B[*a*]P, can migrate into the skin (Paschke *et al.* 2015).

4.3 The aryl hydrocarbon receptor (AHR) as the key enzyme for the metabolisation of PAHs in human cells

The AHR is involved in a large number of different physiological processes in the human organism. These include immune response, apoptosis, skin differentiation and carcinogenesis (Esser *et al.* 2013, Fujii-Kuriyama and Mimura 2005, Hahn 2002, Kawajiri and Fujii-Kuriyama 2007, Nebert *et al.* 1993, Nebert *et al.* 2000, Puga *et al.* 2005, Schlezinger *et al.* 2006). The latter occurs partly through association with xenobiotics such as PAHs, polychlorinated biphenylenes or dioxins, which are also responsible for its identification (Poland and Knutson 1982). Today, a large number of different exogenous (β-NF, polychlorinated biphenyls, PAHs) and endogenous (Kyn, tryptophan derivatives, indole derivatives) ligands are known that

activate the AH receptor and thus trigger shuttling of the receptor from the cytoplasm into the nucleus of the eukaryotic cell (Abel and Haarmann-Stemmann 2010, Denison *et al.* 2011, Sorg 2014). According to the greatly simplified model by Lees & Whitelaw, before activation through ligand binding, the AHR is present in a surplus as a complex with the chaperones XAP2, Hsp90 and p23 in the cytoplasm (Lees and Whitelaw 1999). The ligand-bound AHR translocates into the cell nucleus where it forms a heterodimer with ARNT and is thus capable of associating with specific DNA-binding motifs (*xenobiotic response elements*) in order to trigger the expression of target genes such as *CYP1A1* (Abel and Haarmann-Stemmann 2010, Gasiewicz *et al.* 2008, Lees and Whitelaw 1999). This means that the nucleocytoplasmic translocation of the AHR is necessary to activate the expression of specific genes such as monooxygenases. Very little is known to date about the mechanisms for activation of the AHR, which are closely connected to the intracellular shuttling of the protein. Likewise, little is understood about the physiological relevance and the molecular mechanisms of the nucleocytoplasmic shuttling so far.

To investigate the molecular background of the nucleocytoplasmic translocation of the AHR in greater depth, the N-terminal fluorescence mutant EYFP-AHR was transiently transfected into human HepG2 cells. The constitutive nuclear import of the AHR was confirmed. LMB has no influence on the activity of the AHR and, in thermal stability studies with recombinant AHR, shows no association or stabilisation with the receptor. Nevertheless, after addition of LMB, accumulation of the AHR occurs in the cell nucleus. This is a result of basal AHR shuttling, whereby the addition of LMB has the effect that the AHR protein that has already been constitutively imported cannot be exported to the cytoplasm because of the inhibition, so that it accumulates in the nucleus. The anti-fungal antibiotic isolated from *Streptomyces spp*. inhibits the *chromosomal region maintenance* 1 (CRM1) protein. CRM1, in turn, is responsible for the nuclear export of proteins that have an NES domain, e.g. the AHR. In this way, the data confirm the ligand-independent constitutive nuclear import of the AHR, which was described for the first time by Ikuta *et al*. (Ikuta *et al.* 1998). Import is less pronounced than the ligand-dependent nuclear import, however, because addition of the typical AHR ligand β-NF leads to a marked increase in the kinetics of the nuclear import as compared to the basal translocation of the AHR. Apart from the exogenous ligand β-NF, similar behaviour of the AHR could also be observed after the addition of Kyn. Accordingly, endogenous ligands affect active translocation of the AHR into the cell nucleus after association with the receptor. Export takes place in parallel to maintain the prevailing dominant cytoplasmic compartmentalisation (Richter *et al.* 2001).

Activation of the AHR through β-NF and Kyn is reflected, for example, in the expression of *CYP1A1* and *CYP1B1*. The treatment of cells with LMB did not result in either a nuclear export or an expression of *CYP1A1* and *CYP1B1*. In contrast, both β-NF and Kyn show stabilisation of the complex of recombinant AHR and ligand and thus confirm the increased activity of the AHR nuclear export after treatment with ligands. It was thus possible to show that the import of the AHR is also dependent on endogenous ligands that are produced by the cells themselves and/or excreted by the microbiome in the form of indole and tryptophan derivatives (Jin *et al.* 2014). According to the classic, very static model by Lees & Whitelaw, the AHR is chelated by Hsp90 and the co-chaperones p23 and XAP2. Based on this model, the association with ligands leads to the release of Hsp90, through which the N-terminal bHLH region and thus also the NLS domain are accessible. With this, transport of the AHR into the cell nucleus is finally induced (Lees and Whitelaw 1999). The model indicates that the data acquired is consistent with an accelerated nuclear import of the AHR after addition of ligands. According to Lees & Whitelaw, the interaction between Hsp90 and the bHLH region is very weak so that, under certain circumstances, the NLS domain is only temporarily exposed as a balance of receptor molecules that penetrate the cell nucleus. The hypothesis of the constitutively active NLS domain is supported by the accumulation of deletion mutants in the cell nucleus. Because the NLS region was the only intact region in these mutants, this domain is active independently of further structural elements and constitutively mediates the import of the receptor into the cell nucleus.

Regardless of activation of the AHR through ligands, the receptor is present in a surplus in the cytoplasm. Through activation of the AHR, import of the receptor into the cell nucleus takes place, so that the prevailing balance is disturbed. With a constitutive basal export, this balance with the cytoplasmic surplus of the AHR is restored. This guarantees that the AHR is available for the reaction to incoming exogenous and endogenous ligands. However, this constitutive basal export of the AHR also limits the time interval in which the AHR complex can detect XREs in the promoters of target genes and interact with the DNA. Thus, multiple passages of shuttling are probably required for gene activation.

In addition to the basal export of the AHR, a regulated export of the receptor occurs from the cell nucleus. The AHR has an N-terminal NES domain that is responsible for regulating the translocation. For the murine variant of the AHR, Davarinos & Pollenz were able to measure a significant AHR fraction in the cytoplasm (Davarinos and Pollenz 1999). This AHR showed mutations in the region of the NES domain (Davarinos and Pollenz 1999), while additional

deletion mutants which showed no changes in the region of the NES domain accumulated in the cell nucleus (Pollenz *et al.* 2006). This indicates that the NES domain does not act autonomously, but rather that additional structural elements of the C-terminal domain or additional factors are required to trigger export signals. In order to investigate specifically the influence of the C-terminal domain of the AHR, mutants were selected that showed different deletions at the C-terminus. The identification of valine 647 provided confirmation of the presumption of additional functional elements in the C-terminal region of the AHR suggested in the literature. This means that the export of the AHR is not regulated autonomously by the NES domain, but through interaction with the amino acid valine 647, which is located within the glutamine-rich domain. The function of the NES domain depends on the region of the C-terminal domain within the glutamine-rich domain. The amino acid residue valine 647 is important for the stabilisation of the necessary conformational isomerism for the nuclear export. There was no influence on the nuclear import, because mutations in this region led only to a nuclear localisation of the AHR. The deletion of 201 amino acids at the C-terminal end of the mutant Δ647 resulted in the loss of essential parts of the glutamine-rich domain which are important for the transcriptional activation of target genes through the recruitment of co-activators and components of the basal transcription machinery (Kumar *et al.* 2001). Accordingly, no induction of the gene expression of *CYP1A1* and *CYP1B1* took place. After treatment with the ligands β-NF and LMB, the mutant shows accelerated basal translocation of the AHR into the cell nucleus comparable to the wild type protein, so that the deleted region is not responsible for either the ligand binding or the ligand-based nuclear import of the AHR. Thus, the NLS domain and the ligand binding domain are responsible for ligand-dependent import of the AHR into the cell nucleus without requiring additional structural elements of the C-terminal domain. However, the glutamine-rich domain is necessary for the ligand-dependent inhibition of the AHR export from the cell nucleus, because no import of the AHR mutant into the cell nucleus took place after treatment with the individual ligands. An extension of the mutants from Δ650 – Δ661 showed an increasing fluorescence signal in the cell nucleus. This means that the region connected to valine 647 within the glutamine-rich domain is responsible for the ligand-dependent accumulation of the AHR in the cell nucleus.

To clarify how the dynamic balance of the AHR protein is ultimately maintained, additional deletion mutants in the region of the glutamine-rich domain were analysed that showed deletions of different lengths in the C-terminal domain. With a C-terminal deletion of 125 amino acids, the mutant Δ723 showed an equivalent accumulation of the AHR in the cell

nucleus to mutant Δ647. This means that structural elements in the region of the amino acids 724 – 848 must be responsible for the natural surplus of the AHR in the cytoplasm. With the help of the deletion mutant Δ747, it was possible to show that the prevailing cytoplasmic surplus is restored. The region 723 – 747 is responsible for the formation of the cytoplasmic fraction in connection with the N-terminal NES domain, because when the NES domain was missing in connection with the deletion up to amino acid 723, this led to a significant accumulation of the AHR in the cell nucleus. Autonomous activity of this structural element can be excluded because the fusion of the motif to the C-terminal end of the EYFP did not result in a predominant cytoplasmic accumulation. Furthermore, there was no accumulation of the protein in the cell nucleus after addition of LMB.

The results indicate for the first time that, contrary to the prevailing literature, that regulation of the AHR does not follow a static model; rather, a highly dynamic control of the AHR functionality is in place. C-terminal regions could hereby be identified, such as the amino acid valine 647 and an adjacent region, that play a significant role, both constitutively and ligand-based, in the regulation of the nucleocytoplasmic shuttling of the AHR.

5. Summary

The bacterial degradation of polycyclic aromatic hydrocarbons (PAHs) as environmental contaminants is well described, as is the oxidative formation of potentially toxic intermediates. This led to the hypothesis of commensal PAH-metabolism and, potentially toxification. The skin is the organ most exposed to PAHs. However, the respective metabolism of skin-bacteria has not been investigated and is thus not considered during hazard or risk assessment. Using B[*a*]P as a model substance this project therefore aimed at elucidating the potential role of the skin's microbiome with hindsight to PAH-toxicity.

Carbon-limited enrichment cultures showed microbial B[*a*]P-metabolism to occur ubiquitously, yielding a total of 21 isolates. Phylogenetic analysis identified the corresponding organisms as common skin commensals. All isolates use B[*a*]P as sole source of carbon and energy and degradation was found to be complete as well as partial. In addition, several isolates were found to also grow on other PAHs such as pyrene or fluoranthene. It further appears that degradation of B[*a*]P can proceed via different metabolic pathways. The formation and excretion of toxic metabolites was observed for at least three organisms. One is a complete degrader, the other two metabolise B[*a*]P partially. The respective bacterial culture supernatants showed strong cytotoxic and genotoxic effects when added to human skin or liver cells. For the complete degrader this cytotoxic effect was transient, with the excretion of toxic intermediates being restricted to a time interval of 20-30 hours. In contrast late exponential supernatants of the partial degraders remained cyto- and genotoxic.

Analytical approaches identified a mixture of synergistically operating metabolites. These comprise typical eukaryotic B[*a*]P-diols and B[*a*]P-diones as well as newly identified bacterial metabolites. The latter consist of 5-, 4- and 3-ring systems and include hydroxylated and unmodified B[*a*]A, Phe-4-CO_2H and a B[*a*]P-diol. There are also qualitatively identified B[*a*]P-diols and B[*a*]A-diols.

Bacterial supernatants increase the gene expression of typical monooxygenases of phase I metabolism in eukaryotic skin and liver cells. This is being consonant with the activity of the aryl hydrocarbon receptor. The receptor is important for the activation of further toxification and detoxification reactions. AHR shows a constitutive nucleocytoplasmic shuttling behaviour getting more pronounced while binding of endo- and exogenous ligands. The administration of the physiological functions of the AHR are regulated by a fine tuned regulatory system.

Therefore different N-terminal as well as newly identified C-terminal domains are involved in the regulation of the nucleocytoplasmic AHR shuttling. This domains control ligand-induced and constitutive translocation processes of the AHR. Thereby, for the first time, the molecular background of the time resolved regulation of AHR could be elucidated.

Furthermore, this work was able to show bacterial PAH degradation on the human skin by typical skin commensals. Resulting in a set of secreted cyto- and genotoxic metabolites. Preexisting daily exposure of human skin to PAHs as well as a possible penetration of these metabolites leads to high-risk potential for human carcinogenesis.

6. Zusammenfassung

Im Rahmen dieser Doktorarbeit wurde erstmalig am Beispiel des kanzerogenen B[*a*]P untersucht, ob und in welchem Umfang das menschliche Hautmikrobiom PAKs metabolisiert und inwieweit sich daraus Bedenken für den Wirt ergeben.

Ausgehend von Hautabstrichen unterschiedlicher Probanden wurden 21 verschiedene B[*a*]P-abbauende Bakterien isoliert und hinsichtlich ihres B[*a*]P-metabolisierenden Potenzials untersucht. Identifiziert wurden vorwiegend typische Hautkommensalen, wobei der Abbau von B[*a*]P als alleinige Kohlenstoff- und Energiequelle vollständig oder partiell erfolgte. Daten zur Biochemie deuten dabei auf die Existenz mehrerer verschiedener Abbauwege hin, während Substrattests zeigen, dass außer B[*a*]P auch andere hochmolekulare PAKs verwertet werden können. Dabei bildet ein Teil der Organismen während des Abbaus Intermediate, die ausgeschieden werden und in zellulären Assays auf Humanzellen stark zytotoxisch und genotoxisch wirken. In diesem Zusammenhang deuten sowohl die bakteriellen Wachstumsraten als auch die Toxizitätstests auf ein 20-30 stündiges Exkretionsfenster für die toxischen Metabolite hin. Mittels unterschiedlicher analytischer Methoden konnte gezeigt werden, dass es sich bei den ausgeschiedenen Intermediaten um ein synergistisch wirkendes Gemisch von bereits aus dem eukaryotischen Stoffwechsel bekannten hydroxylierten B[*a*]Ps und B[*a*]P-Dionen, sowie bisher unbekannten typisch bakteriellen Intermediaten handelt. Letztere reichen von 3- bis zu 5-Ringsystemen und umfassen unter anderem hydroxyliertes und nicht-hydroxyliertes B[*a*]A, Phe-4-CO_2H und ein bisher nicht näher identifizierbares B[*a*]A-diol, sowie qualitativ identifizierte B[*a*]P-ole und B[*a*]A-diole. Vergleichende Toxizitätsstudien mit bakteriellen Überständen und Mischungen der identifizierten Metabolite belegen, dass weitere zyto- und genotoxische Intermediate exkretiert werden, die bisher nicht identifiziert werden konnten.

Anhand der bakteriellen Überstände ist erkennbar, dass Monooxygenasen des Phase I-Stoffwechsels, über den AHR aktiviert werden um weitere Giftungs- und Entgiftungsreaktionen in der Zelle zu aktivieren. Zur Ausübung der Funktionalität besitzt der AHR ein fein austariertes regulatorisches System des nukleocytoplasmatischen shuttlings. Verschiedene N-terminale, sowie neu identifizierte C-terminale Bereiche sind maßgeblich an der Regulation einer basalen Translokation sowie eines Liganden gestützten shuttling durch exogene (β-NF, PAKs, PCBs) und endogene Liganden (Kynurenin, mikrobielle Tryptophan- und Indolderivate) beteiligt.

Damit wurde ein wichtiger Beitrag zu den molekularbiologischen Hintergründen der Aktivität des AHR und der Regulation seiner Funktionen aufgezeigt.

Des Weiteren zeigt diese Arbeit erstmals, dass der bakterielle Abbau von PAKs am Beispiel des B[*a*]Ps auf der Haut existiert. Durch die Sekretion zytotoxischer, als auch genotoxischer Metabolite stellt das humane Hautmikrobiom somit bei gegebener Exposition gegenüber PAKs, sowie gegebener Penetration ein Risiko für den Menschen dar.

7. Literature

Abel, J. and T. Haarmann-Stemmann (2010). "An introduction to the molecular basics of aryl hydrocarbon receptor biology." Biol Chem 391(11): 1235-1248.

Aitken, M. D., W. T. Stringfellow, R. D. Nagel, C. Kazunga and S. H. Chen (1998). "Characteristics of phenanthrene-degrading bacteria isolated from soils contaminated with polycyclic aromatic hydrocarbons." Can J Microbiol 44(8): 743-752.

Aldag, C., D. Nogueira Teixeira and P. S. Leventhal (2016). "Skin rejuvenation using cosmetic products containing growth factors, cytokines, and matrikines: a review of the literature." Clinical, Cosmetic and Investigational Dermatology 9: 411-419.

Anderson, C., A. Hehr, R. Robbins, R. Hasan, M. Athar, H. Mukhtar and C. A. Elmets (1995). "Metabolic requirements for induction of contact hypersensitivity to immunotoxic polyaromatic hydrocarbons." J Immunol 155(7): 3530-3537.

Anderson, R. T. and D. R. Lovley (1999). "Naphthalene and Benzene Degradation under Fe(III)-Reducing Conditions in Petroleum-Contaminated Aquifers." Bioremediation Journal 3(2): 121-135.

Arumugam, M., J. Raes, E. Pelletier, D. Le Paslier, T. Yamada, D. R. Mende, G. R. Fernandes, J. Tap, T. Bruls, J. M. Batto, M. Bertalan, N. Borruel, F. Casellas, L. Fernandez, L. Gautier, T. Hansen, M. Hattori, T. Hayashi, M. Kleerebezem, K. Kurokawa, M. Leclerc, F. Levenez, C. Manichanh, H. B. Nielsen, T. Nielsen, N. Pons, J. Poulain, J. Qin, T. Sicheritz-Ponten, S. Tims, D. Torrents, E. Ugarte, E. G. Zoetendal, J. Wang, F. Guarner, O. Pedersen, W. M. de Vos, S. Brunak, J. Dore, M. Antolin, F. Artiguenave, H. M. Blottiere, M. Almeida, C. Brechot, C. Cara, C. Chervaux, A. Cultrone, C. Delorme, G. Denariaz, R. Dervyn, K. U. Foerstner, C. Friss, M. van de Guchte, E. Guedon, F. Haimet, W. Huber, J. van Hylckama-Vlieg, A. Jamet, C. Juste, G. Kaci, J. Knol, O. Lakhdari, S. Layec, K. Le Roux, E. Maguin, A. Merieux, R. Melo Minardi, C. M'Rini, J. Muller, R. Oozeer, J. Parkhill, P. Renault, M. Rescigno, N. Sanchez, S. Sunagawa, A. Torrejon, K. Turner, G. Vandemeulebrouck, E. Varela, Y. Winogradsky, G. Zeller, J. Weissenbach, S. D. Ehrlich and P. Bork (2011). "Enterotypes of the human gut microbiome." Nature 473(7346): 174-180.

Baboshin, M. A. and L. A. Golovleva (2012). "Biodegradation of polycyclic aromatic hydrocarbons (PAH) by aerobic bacteria and its kinetics aspects." Mikrobiologiia 81(6): 695-706.

Backhed, F. (2012). "Host responses to the human microbiome." Nutr Rev 70 Suppl 1: S14-17.

Backhed, F., R. E. Ley, J. L. Sonnenburg, D. A. Peterson and J. I. Gordon (2005). "Host-bacterial mutualism in the human intestine." Science 307(5717): 1915-1920.

Baird, W. M., L. A. Hooven and B. Mahadevan (2005). "Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action." Environ Mol Mutagen 45(2-3): 106-114.

Bamforth, S. M. and I. Singleton (2005). "Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions." Journal of Chemical Technology & Biotechnology 80(7): 723-736.

Barouki, R., M. Aggerbeck, L. Aggerbeck and X. Coumoul (2012). "The aryl hydrocarbon receptor system." Drug Metabol Drug Interact 27(1): 3-8.

Bauer, E., Z. Guo, Y. F. Ueng, L. C. Bell, D. Zeldin and F. P. Guengerich (1995). "Oxidation of benzo[*a*]pyrene by recombinant human cytochrome P450 enzymes." Chem Res Toxicol 8(1): 136-142.

Beatty, J. K., S. V. Akierman, J. P. Motta, S. Muise, M. L. Workentine, J. J. Harrison, A. Bhargava, P. L. Beck, K. P. Rioux, G. W. McKnight, J. L. Wallace and A. G. Buret (2017). "Giardia duodenalis induces pathogenic dysbiosis of human intestinal microbiota biofilms." Int J Parasitol.

Behrens, A., K. Schirmer, N. C. Bols and H. Segner (2001). "Polycyclic aromatic hydrocarbons as inducers of cytochrome P4501A enzyme activity in the rainbow trout liver cell line, RTL-W1, and in primary cultures of rainbow trout hepatocytes." Environ Toxicol Chem 20(3): 632-643.

Bergstrom, A., T. H. Skov, M. I. Bahl, H. M. Roager, L. B. Christensen, K. T. Ejlerskov, C. Molgaard, K. F. Michaelsen and T. R. Licht (2014). "Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants." Appl Environ Microbiol 80(9): 2889-2900.

Bessede, A., M. Gargaro, M. T. Pallotta, D. Matino, G. Servillo, C. Brunacci, S. Bicciato, E. M. Mazza, A. Macchiarulo, C. Vacca, R. Iannitti, L. Tissi, C. Volpi, M. L. Belladonna, C. Orabona, R. Bianchi, T. V. Lanz, M. Platten, M. A. Della Fazia, D. Piobbico, T. Zelante, H. Funakoshi, T. Nakamura, D. Gilot, M. S. Denison, G. J. Guillemin, J. B. DuHadaway, G. C. Prendergast, R. Metz, M. Geffard, L. Boon, M. Pirro, A. Iorio, B. Veyret, L. Romani, U. Grohmann, F. Fallarino and P. Puccetti (2014). "Aryl hydrocarbon receptor control of a disease tolerance defence pathway." Nature 511(7508): 184-190.

BfR (2009). “PAK in verbrauchernahen Produkten müssen soweit wie möglich reduziert werden” Stellungnahme Nr. 025/2009

BfR (2009). “Polyzyklische aromatische Kohlenwasserstoffe (PAK) in Spielzeug” Stellungnahme Nr. 051/2009

BfR (2010). “Krebserzeugende polyzyklische aromatische Kohlenwasserstoffe (PAK) in Verbraucherprodukten sollen EU-weit reguliert werden - Risikobewertung des BfR im Rahmen eines Beschränkungsvorschlages unter REACH“ Stellungnahme Nr. 032/2010

Boffetta, P., N. Jourenkova and P. Gustavsson (1997). "Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons." Cancer Causes Control 8(3): 444-472.

Boukamp, P., R. T. Petrussevska, D. Breitkreutz, J. Hornung, A. Markham and N. E. Fusenig (1988). "Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line." The Journal of Cell Biology 106(3): 761-771.

Braga, R. M., M. N. Dourado and W. L. Araujo (2016). "Microbial interactions: ecology in a molecular perspective." Braz J Microbiol 47 Suppl 1: 86-98.

Bravo, J. A., P. Forsythe, M. V. Chew, E. Escaravage, H. M. Savignac, T. G. Dinan, J. Bienenstock and J. F. Cryan (2011). "Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve." Proc Natl Acad Sci U S A 108(38): 16050-16055.

Brezna, B., A. A. Khan and C. E. Cerniglia (2003). "Molecular characterization of dioxygenases from polycyclic aromatic hydrocarbon-degrading *Mycobacterium spp*." FEMS Microbiol Lett 223(2): 177-183.

Brezna, B., O. Kweon, R. L. Stingley, J. P. Freeman, A. A. Khan, B. Polek, R. C. Jones and C. E. Cerniglia (2006). "Molecular characterization of cytochrome P450 genes in the polycyclic aromatic hydrocarbon degrading *Mycobacterium vanbaalenii* PYR-1." Appl Microbiol Biotechnol 71(4): 522-532.

Brinkmann, J., K. Stolpmann, S. Trappe, T. Otter, D. Genkinger, U. Bock, M. Liebsch, F. Henkler, C. Hutzler and A. Luch (2013). "Metabolically competent human skin models: activation and genotoxicity of benzo[*a*]pyrene." Toxicol Sci 131(2): 351-359.

Budavari, S. (1989). The Merck index : an encyclopedia of chemicals, drugs, and biologicals. Rahway, N.J., U.S.A., Merck.

Burczynski, M. E. and T. M. Penning (2000). "Genotoxic polycyclic aromatic hydrocarbon ortho-quinones generated by aldo-keto reductases induce *CYP1A1* via nuclear translocation of the aryl hydrocarbon receptor." Cancer Res 60(4): 908-915.

Butnaru, C. A. and J. Kanitakis (2002). "Structure of normal human skin." Eur J Dermatol 12(6): II-IV.

Cajthaml, T., M. Moder, P. Kacer, V. Sasek and P. Popp (2002). "Study of fungal degradation products of polycyclic aromatic hydrocarbons using gas chromatography with ion trap mass spectrometry detection." J Chromatogr A 974(1-2): 213-222.

Callewaert, C., P. Hutapea, T. Van de Wiele and N. Boon (2014). "Deodorants and antiperspirants affect the axillary bacterial community." Arch Dermatol Res 306(8): 701-710.

Callewaert, C., F. M. Kerckhof, M. S. Granitsiotis, M. Van Gele, T. Van de Wiele and N. Boon (2013). "Characterization of *Staphylococcus* and *Corynebacterium* clusters in the human axillary region." PLoS One 8(8): e70538.

Cani, P. D., A. Everard and T. Duparc (2013). "Gut microbiota, enteroendocrine functions and metabolism." Curr Opin Pharmacol 13(6): 935-940.

Capone, K. A., S. E. Dowd, G. N. Stamatas and J. Nikolovski (2011). "Diversity of the human skin microbiome early in life." J Invest Dermatol 131(10): 2026-2032.

Cavalieri, E. L., E. G. Rogan, K. M. Li, R. Todorovic, F. Ariese, R. Jankowiak, N. Grubor and G. J. Small (2005). "Identification and quantification of the depurinating DNA adducts formed in mouse skin treated with dibenzo[*a,l*]pyrene (DB[*a,l*]P) or its metabolites and in rat mammary gland treated with DB[*a,l*]P." Chem Res Toxicol 18(6): 976-983.

Cerniglia, C. E. (1992). "Biodegradation of polycyclic aromatic hydrocarbons." Biodegradation 3(2): 351-368.

Cerniglia, C. E. (1997). "Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation." J Ind Microbiol Biotechnol 19(5-6): 324-333.

Cerniglia, C. E. and M. A. Heitkamp (1990). "Polycyclic aromatic hydrocarbon degradation by *Mycobacterium*." Methods in Enzymology 188: 148-153.

Chang, B. V., I. T. Chang and S. Y. Yuan (2008). "Anaerobic degradation of phenanthrene and pyrene in mangrove sediment." Bull Environ Contam Toxicol 80(2): 145-149.

Chang, B. V., L. C. Shiung and S. Y. Yuan (2002). "Anaerobic biodegradation of polycyclic aromatic hydrocarbon in soil." Chemosphere 48(7): 717-724.

Chang, J. S., D. K. Cha, M. Radosevich and Y. Jin (2015). "Effects of biosurfactant-producing bacteria on biodegradation and transport of phenanthrene in subsurface soil." J Environ Sci Health A Tox Hazard Subst Environ Eng 50(6): 611-616.

Christensen, G. J. and H. Bruggemann (2014). "Bacterial skin commensals and their role as host guardians." Benef Microbes 5(2): 201-215.

Clarke, G., R. M. Stilling, P. J. Kennedy, C. Stanton, J. F. Cryan and T. G. Dinan (2014). "Minireview: Gut microbiota: the neglected endocrine organ." Mol Endocrinol 28(8): 1221-1238.

Clemente, A. R., T. A. Anazawa and L. R. Durrant (2001). "Biodegradation of polycyclic aromatic hydrocarbons by soil fungi." Brazilian Journal of Microbiology 32: 255-261.

Cogen, A. L., K. Yamasaki, J. Muto, K. M. Sanchez, L. Crotty Alexander, J. Tanios, Y. Lai, J. E. Kim, V. Nizet and R. L. Gallo (2010). "*Staphylococcus epidermidis* antimicrobial delta-toxin (phenol-soluble modulin-gamma) cooperates with host antimicrobial peptides to kill group A *Streptococcus*." PLoS One 5(1): e8557.

Commins, B. T. (1969). "Formation of polycyclic aromatic hydrocarbons during pyrolysis and combustion of hydrocarbons." Atmos Environ 3(5): 565-572.

Conney, A. H. (1982). "Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture." Cancer Res 42(12): 4875-4917.

Costello, E. K., C. L. Lauber, M. Hamady, N. Fierer, J. I. Gordon and R. Knight (2009). "Bacterial community variation in human body habitats across space and time." Science 326(5960): 1694-1697.

Costello, E. K., K. Stagaman, L. Dethlefsen, B. J. Bohannan and D. A. Relman (2012). "The application of ecological theory toward an understanding of the human microbiome." Science 336(6086): 1255-1262.

Creusot, N., F. Brion, B. Piccini, H. Budzinski, J. M. Porcher and S. Ait-Aissa (2015). "BFCOD activity in fish cell lines and zebrafish embryos and its modulation by chemical ligands of human aryl hydrocarbon and nuclear receptors." Environ Sci Pollut Res Int 22(21): 16393-16404.

Cryan, J. F. and S. M. O'Mahony (2011). "The microbiome-gut-brain axis: from bowel to behavior." Neurogastroenterol Motil 23(3): 187-192.

Danyi, S., F. Brose, C. Brasseur, Y. J. Schneider, Y. Larondelle, L. Pussemier, J. Robbens, S. De Saeger, G. Maghuin-Rogister and M. L. Scippo (2009). "Analysis of EU priority polycyclic aromatic hydrocarbons in food supplements using high performance liquid chromatography coupled to an ultraviolet, diode array or fluorescence detector." Anal Chim Acta 633(2): 293-299.

Davarinos, N. A. and R. S. Pollenz (1999). "Aryl hydrocarbon receptor imported into the nucleus following ligand binding is rapidly degraded via the cytosplasmic proteasome following nuclear export." J Biol Chem 274(40): 28708-28715.

De Filippo, C., D. Cavalieri, M. Di Paola, M. Ramazzotti, J. B. Poullet, S. Massart, S. Collini, G. Pieraccini and P. Lionetti (2010). "Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa." Proc Natl Acad Sci U S A 107(33): 14691-14696.

Dekant, W. (2009). "The role of biotransformation and bioactivation in toxicity." EXS 99: 57-86.

Denison, M. S., A. A. Soshilov, G. He, D. E. DeGroot and B. Zhao (2011). "Exactly the same but different: promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor." Toxicol Sci 124(1): 1-22.

Dethlefsen, L., S. Huse, M. L. Sogin and D. A. Relman (2008). "The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing." PLoS Biol 6(11): e280.

Devaraj, S., P. Hemarajata and J. Versalovic (2013). "The human gut microbiome and body metabolism: implications for obesity and diabetes." Clin Chem 59(4): 617-628.

Ding, T. and P. D. Schloss (2014). "Dynamics and associations of microbial community types across the human body." Nature 509(7500): 357-360.

Dominguez-Bello, M. G., E. K. Costello, M. Contreras, M. Magris, G. Hidalgo, N. Fierer and R. Knight (2010). "Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns." Proc Natl Acad Sci U S A 107(26): 11971-11975.

Eaton, R. W. and P. J. Chapman (1992). "Bacterial metabolism of naphthalene: construction and use of recombinant bacteria to study ring cleavage of 1,2-dihydroxynaphthalene and subsequent reactions." J Bacteriol 174(23): 7542-7554.

EFSA (2008). „Polycyclic aromatic hydrocarbons in food. Scientific Opinion of the panel of contaminants in the food chain.“ The EFSA Journal 724, 1-114

Elias, P. M. (1981). "Epidermal Lipids, Membranes, and Keratinization." International Journal of Dermatology 20(1): 1-19.

Ellis, H. (1981). "Microbiology of Human Skin. W. C. Noble. Second edition." British Journal of Surgery 68(11): 828-828.

ElRakaiby, M., B. E. Dutilh, M. R. Rizkallah, A. Boleij, J. N. Cole and R. K. Aziz (2014). "Pharmacomicrobiomics: the impact of human microbiome variations on systems pharmacology and personalized therapeutics." OMICS 18(7): 402-414.

Enright, E. F., C. G. Gahan, S. A. Joyce and B. T. Griffin (2016). "The Impact of the Gut Microbiota on Drug Metabolism and Clinical Outcome." Yale J Biol Med 89(3): 375-382.

Esser, C., I. Bargen, H. Weighardt, T. Haarmann-Stemmann and J. Krutmann (2013). "Functions of the aryl hydrocarbon receptor in the skin." Semin Immunopathol 35(6): 677-691.

Esser, C., A. Rannug and B. Stockinger (2009). "The aryl hydrocarbon receptor in immunity." Trends Immunol 30(9): 447-454.

Faergemann, J. and O. Larko (1987). "The effect of UV-light on human skin microorganisms." Acta Derm Venereol 67(1): 69-72.

Feng, X., W. Pisula and K. Müllen (2009). Large polycyclic aromatic hydrocarbons: Synthesis and discotic organization. Pure and Applied Chemistry. 81: 2203.

Fernandez-Salguero, P. M., J. M. Ward, J. P. Sundberg and F. J. Gonzalez (1997). "Lesions of aryl-hydrocarbon receptor-deficient mice." Vet Pathol 34(6): 605-614.

Fetzer, J. C. (2007). "The chemistra and analysis of large PAHs." Polycyclic Aromatic Compounds 27(2): 143-162.

Fierer, N., M. Hamady, C. L. Lauber and R. Knight (2008). "The influence of sex, handedness, and washing on the diversity of hand surface bacteria." Proc Natl Acad Sci U S A 105(46): 17994-17999.

Fierer, N., C. L. Lauber, N. Zhou, D. McDonald, E. K. Costello and R. Knight (2010). "Forensic identification using skin bacterial communities." Proc Natl Acad Sci U S A 107(14): 6477-6481.

Findley, K. and E. A. Grice (2014). "The skin microbiome: a focus on pathogens and their association with skin disease." PLoS Pathog 10(10): e1004436.

Fitch, M. E., I. V. Cross and J. M. Ford (2003). "p53 responsive nucleotide excision repair gene products p48 and XPC, but not p53, localize to sites of UV-irradiation-induced DNA damage, *in vivo*." Carcinogenesis 24(5): 843-850.

Forsythe, P. and J. Bienenstock (2010). "Immunomodulation by commensal and probiotic bacteria." Immunol Invest 39(4-5): 429-448.

Freter, R. and A. Ozawa (1963). "Explanation for limitation of populations of *Escherichia coli* in broth cultures." J Bacteriol 86: 904-910.

Fujii-Kuriyama, Y. and J. Mimura (2005). "Molecular mechanisms of AhR functions in the regulation of cytochrome P450 genes." Biochem Biophys Res Commun 338(1): 311-317.

Gallo, R. L., M. Murakami, T. Ohtake and M. Zaiou (2002). "Biology and clinical relevance of naturally occurring antimicrobial peptides." J Allergy Clin Immunol 110(6): 823-831.

Gao, Z., C. H. Tseng, Z. Pei and M. J. Blaser (2007). "Molecular analysis of human forearm superficial skin bacterial biota." Proc Natl Acad Sci U S A 104(8): 2927-2932.

Gasiewicz, T. A., E. C. Henry and L. L. Collins (2008). "Expression and activity of aryl hydrocarbon receptors in development and cancer." Crit Rev Eukaryot Gene Expr 18(4): 279-321.

Ghosh, A., S. A. Chaudhary, S. R. Apurva, T. Tiwari, S. Gupta, A. K. Singh, K. H. Katudia, M. P. Patel and S. K. Chikara (2013). "Whole-Genome Sequencing of *Micrococcus luteus* Strain Modasa, of Indian Origin." Genome Announc 1(2): e0007613.

Giacomoni, P. U., T. Mammone and M. Teri (2009). "Gender-linked differences in human skin." J Dermatol Sci 55(3): 144-149.

Gianfreda, L., F. Xu and J.-M. Bollag (1999). "Laccases: A Useful Group of Oxidoreductive Enzymes." Bioremediation Journal 3(1): 1-26.

Gibson, D. T. (1999). "*Beijerinckia* sp. strain B1: a strain by any other name." Journal of Industrial Microbiology and Biotechnology 23(4-5): 284-293.

Gibson, D. T., V. Mahadevan, D. M. Jerina, H. Yogi and H. J. Yeh (1975). "Oxidation of the carcinogens benzo[*a*]pyrene and benzo[*a*]anthracene to dihydrodiols by a bacterium." Science 189(4199): 295-297.

Grice, E. A. (2014). "The skin microbiome: potential for novel diagnostic and therapeutic approaches to cutaneous disease." Semin Cutan Med Surg 33(2): 98-103.

Grice, E. A., H. H. Kong, S. Conlan, C. B. Deming, J. Davis, A. C. Young, G. G. Bouffard, R. W. Blakesley, P. R. Murray, E. D. Green, M. L. Turner and J. A. Segre (2009). "Topographical and temporal diversity of the human skin microbiome." Science 324(5931): 1190-1192.

Grice, E. A., H. H. Kong, G. Renaud, A. C. Young, G. G. Bouffard, R. W. Blakesley, T. G. Wolfsberg, M. L. Turner and J. A. Segre (2008). "A diversity profile of the human skin microbiota." Genome Res 18(7): 1043-1050.

Grice, E. A. and J. A. Segre (2012). "The human microbiome: our second genome." Annu Rev Genomics Hum Genet 13: 151-170.

Hahn, M. E. (2002). "Aryl hydrocarbon receptors: diversity and evolution." Chem Biol Interact 141(1-2): 131-160.

Hahn, M. E., L. L. Allan and D. H. Sherr (2009). "Regulation of constitutive and inducible AHR signaling: Complex interactions involving the AHR repressor." Biochemical Pharmacology 77(4): 485-497.

Haiser, H. J., D. B. Gootenberg, K. Chatman, G. Sirasani, E. P. Balskus and P. J. Turnbaugh (2013). "Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*." Science 341(6143): 295-298.

Haiser, H. J., K. L. Seim, E. P. Balskus and P. J. Turnbaugh (2014). "Mechanistic insight into digoxin inactivation by Eggerthella lenta augments our understanding of its pharmacokinetics." Gut Microbes 5(2): 233-238.

Haiser, H. J. and P. J. Turnbaugh (2013). "Developing a metagenomic view of xenobiotic metabolism." Pharmacol Res 69(1): 21-31.

Hannigan, G. D. and E. A. Grice (2013). "Microbial ecology of the skin in the era of metagenomics and molecular microbiology." Cold Spring Harb Perspect Med 3(12): a015362.

Haritash, A. K. and C. P. Kaushik (2009). "Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review." J Hazard Mater 169(1-3): 1-15.

Harms, H., D. Schlosser and L. Y. Wick (2011). "Untapped potential: exploiting fungi in bioremediation of hazardous chemicals." Nat Rev Microbiol 9(3): 177-192.

He, Y., A. Yediler, T. Sun and A. Kettrup (1995). "Adsorption of fluoranthene on soil and lava: Effects of the organic carbon contents of adsorbents and temperature." Chemosphere 30(1): 141-150.

Hecht, S. S. (2002). "Tobacco smoke carcinogens and breast cancer." Environ Mol Mutagen 39(2-3): 119-126.

Heitkamp, M. A. and C. E. Cerniglia (1988). "Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil field." Appl Environ Microbiol 54(6): 1612-1614.

Heitkamp, M. A., J. P. Freeman, D. W. Miller and C. E. Cerniglia (1988). "Pyrene degradation by a *Mycobacterium sp*.: identification of ring oxidation and ring fission products." Appl Environ Microbiol 54(10): 2556-2565.

Hofrichter, M., K. Scheibner, I. Schneegass and W. Fritsche (1998). "Enzymatic Combustion of Aromatic and Aliphatic Compounds by Manganese Peroxidase from *Nematoloma frowardii*." Appl Environ Microbiol 64(2): 399-404.

Holick, M. F. (1995). "Environmental factors that influence the cutaneous production of vitamin D." Am J Clin Nutr 61(3 Suppl): 638S-645S.

Hollstein, M., J. McCann, F. A. Angelosanto and W. W. Nichols (1979). "Short-term tests for carcinogens and mutagens." Mutat Res 65(3): 133-226.

Hsiao, E. Y., S. W. McBride, S. Hsien, G. Sharon, E. R. Hyde, T. McCue, J. A. Codelli, J. Chow, S. E. Reisman, J. F. Petrosino, P. H. Patterson and S. K. Mazmanian (2013). "Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders." Cell 155(7): 1451-1463.

Ikuta, T., H. Eguchi, T. Tachibana, Y. Yoneda and K. Kawajiri (1998). "Nuclear localization and export signals of the human aryl hydrocarbon receptor." J Biol Chem 273(5): 2895-2904.

Ishizaki, A., K. Saito, N. Hanioka, S. Narimatsu and H. Kataoka (2010). "Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high-performance liquid chromatography-fluorescence detection." J Chromatogr A 1217(35): 5555-5563.

Iwase, T., Y. Uehara, H. Shinji, A. Tajima, H. Seo, K. Takada, T. Agata and Y. Mizunoe (2010). "*Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization." Nature 465(7296): 346-349.

Jarvis, W. R. (1994). "Handwashing--the Semmelweis lesson forgotten?" Lancet 344(8933): 1311-1312.

Jiang, H., S. L. Gelhaus, D. Mangal, R. G. Harvey, I. A. Blair and T. M. Penning (2007). "Metabolism of benzo[*a*]pyrene in human bronchoalveolar H358 cells using liquid chromatography-mass spectrometry." Chem Res Toxicol 20(9): 1331-1341.

Jin, U. H., S. O. Lee, G. Sridharan, K. Lee, L. A. Davidson, A. Jayaraman, R. S. Chapkin, R. Alaniz and S. Safe (2014). "Microbiome-derived tryptophan metabolites and their aryl hydrocarbon receptor-dependent agonist and antagonist activities." Mol Pharmacol 85(5): 777-788.

Johnsen, A. R., L. Y. Wick and H. Harms (2005). "Principles of microbial PAH-degradation in soil." Environ Pollut 133(1): 71-84.

Jost, T., C. Lacroix, C. Braegger and C. Chassard (2015). "Impact of human milk bacteria and oligosaccharides on neonatal gut microbiota establishment and gut health." Nutrition Reviews 73(7): 426-437.

Jourova, L., P. Anzenbacher and E. Anzenbacherova (2016). "Human gut microbiota plays a role in the metabolism of drugs." Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 160(3): 317-326.

Juhasz, A. L., M. L. Britz and G. A. Stanley (1996). "Degradation of high molecular weight polycyclic aromatic hydrocarbons by *Pseudomonas cepacia*." Biotechnology Letters 18(5): 577-582.

Juhasz, A. L. and R. Naidu (2000). "Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[*a*]pyrene." International Biodeterioration & Biodegradation 45(1–2): 57-88.

Kanitakis, J. (2002). "Anatomy, histology and immunohistochemistry of normal human skin." Eur J Dermatol 12(4): 390-399; quiz 400-391.

Kaplan, D. H., A. Kissenpfennig and B. E. Clausen (2008). "Insights into Langerhans cell function from Langerhans cell ablation models." Eur J Immunol 38(9): 2369-2376.

Karzel, K. and R. K. Liedtke (1989). "Mechanisms of transcutaneous absorption. Pharmacologic and biochemical aspects." Arzneimittelforschung 39(11A): 1487-1491.

Kastan, M. B., Q. Zhan, W. S. el-Deiry, F. Carrier, T. Jacks, W. V. Walsh, B. S. Plunkett, B. Vogelstein and A. J. Fornace, Jr. (1992). "A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia." Cell 71(4): 587-597.

Kästner, M., M. Breuer-Jammali and B. Mahro (1998). "Impact of inoculation protocols, salinity, and pH on the degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil." Appl Environ Microbiol 64(1): 359-362.

Kawajiri, K. and Y. Fujii-Kuriyama (2007). "Cytochrome P450 gene regulation and physiological functions mediated by the aryl hydrocarbon receptor." Arch Biochem Biophys 464(2): 207-212.

Keith, L. H. (2015). "The Source of U.S. EPA's Sixteen PAH Priority Pollutants." Polycyclic Aromatic Compounds 35(2-4): 147-160.

Khan, A. A., S. J. Kim, D. D. Paine and C. E. Cerniglia (2002). "Classification of a polycyclic aromatic hydrocarbon-metabolizing bacterium, *Mycobacterium sp.* strain PYR-1, as *Mycobacterium vanbaalenii sp. nov*." Int J Syst Evol Microbiol 52(Pt 6): 1997-2002.

Khan, A. A., R. F. Wang, W. W. Cao, D. R. Doerge, D. Wennerstrom and C. E. Cerniglia (2001). "Molecular cloning, nucleotide sequence, and expression of genes encoding a polycyclic aromatic ring dioxygenase from *Mycobacterium sp*. strain PYR-1." Appl Environ Microbiol 67(8): 3577-3585.

Kim, J. H., K. H. Stansbury, N. J. Walker, M. A. Trush, P. T. Strickland and T. R. Sutter (1998). "Metabolism of benzo[*a*]pyrene and benzo[*a*]pyrene-7,8-diol by human cytochrome P450 1B1." Carcinogenesis 19(10): 1847-1853.

Kim, S.-J., O. Kweon and C. E. Cerniglia (2010). Degradation of Polycyclic Aromatic Hydrocarbons by *Mycobacterium* Strains. Handbook of Hydrocarbon and Lipid Microbiology. K. N. Timmis. Berlin, Heidelberg, Springer Berlin Heidelberg: 1865-1879.

Kim, S. J., R. C. Jones, C. J. Cha, O. Kweon, R. D. Edmondson and C. E. Cerniglia (2004). "Identification of proteins induced by polycyclic aromatic hydrocarbon in *Mycobacterium vanbaalenii* PYR-1 using two-dimensional polyacrylamide gel electrophoresis and *de novo* sequencing methods." Proteomics 4(12): 3899-3908.

Kim, S. J., O. Kweon, J. P. Freeman, R. C. Jones, M. D. Adjei, J. W. Jhoo, R. D. Edmondson and C. E. Cerniglia (2006). "Molecular cloning and expression of genes encoding a novel dioxygenase involved in low- and high-molecular-weight polycyclic aromatic hydrocarbon degradation in *Mycobacterium vanbaalenii* PYR-1." Appl Environ Microbiol 72(2): 1045-1054.

Kim, S. J., O. Kweon, R. C. Jones, R. D. Edmondson and C. E. Cerniglia (2008). "Genomic analysis of polycyclic aromatic hydrocarbon degradation in *Mycobacterium vanbaalenii* PYR-1." Biodegradation 19(6): 859-881.

Kim, S. J., O. Kweon, R. C. Jones, J. P. Freeman, R. D. Edmondson and C. E. Cerniglia (2007). "Complete and integrated pyrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1 based on systems biology." J Bacteriol 189(2): 464-472.

Kim, Y. H., K. H. Engesser and C. E. Cerniglia (2003). "Two polycyclic aromatic hydrocarbon *o*-quinone reductases from a pyrene-degrading *Mycobacterium*." Arch Biochem Biophys 416(2): 209-217.

Kim, Y. H., K. H. Engesser and C. E. Cerniglia (2005). "Numerical and genetic analysis of polycyclic aromatic hydrocarbon-degrading *mycobacteria*." Microb Ecol 50(1): 110-119.

Kim, Y. H., J. P. Freeman, J. D. Moody, K. H. Engesser and C. E. Cerniglia (2005). "Effects of pH on the degradation of phenanthrene and pyrene by *Mycobacterium vanbaalenii* PYR-1." Appl Microbiol Biotechnol 67(2): 275-285.

Kim, Y. H., J. D. Moody, J. P. Freeman, B. Brezna, K. H. Engesser and C. E. Cerniglia (2004). "Evidence for the existence of PAH-quinone reductase and catechol-*O*-methyltransferase in *Mycobacterium vanbaalenii* PYR-1." J Ind Microbiol Biotechnol 31(11): 507-516.

Kimura, N., W. Kitagawa, T. Mori, N. Nakashima, T. Tamura and Y. Kamagata (2006). "Genetic and biochemical characterization of the dioxygenase involved in lateral dioxygenation of dibenzofuran from *Rhodococcus opacus* strain SAO101." Applied Microbiology and Biotechnology 73(2): 474-484.

Ko, H. P., S. T. Okino, Q. Ma and J. P. Whitlock, Jr. (1997). "Transactivation domains facilitate promoter occupancy for the dioxin-inducible *CYP1A1* gene *in vivo*." Mol Cell Biol 17(7): 3497-3507.

Kollias, N., R. M. Sayre, L. Zeise and M. R. Chedekel (1991). "Photoprotection by melanin." J Photochem Photobiol B 9(2): 135-160.

Kuk, S., Y. Uyar, S. Karaca and S. Yazar (2016). "Microbiota: In Health and in Sickness, From Birth to Death." Turkiye Parazitol Derg 40(2): 97-106.

Kumar, M. B., P. Ramadoss, R. K. Reen, J. P. Vanden Heuvel and G. H. Perdew (2001). "The Q-rich subdomain of the human Ah receptor transactivation domain is required for dioxin-mediated transcriptional activity." J Biol Chem 276(45): 42302-42310.

Kweon, O., S. J. Kim, S. Baek, J. C. Chae, M. D. Adjei, D. H. Baek, Y. C. Kim and C. E. Cerniglia (2008). "A new classification system for bacterial Rieske non-heme iron aromatic ring-hydroxylating oxygenases." BMC Biochem 9: 11.

Kweon, O., S. J. Kim, J. Blom, S. K. Kim, B. S. Kim, D. H. Baek, S. I. Park, J. B. Sutherland and C. E. Cerniglia (2015). "Comparative functional pan-genome analyses to build connections between genomic dynamics and phenotypic evolution in polycyclic aromatic hydrocarbon metabolism in the genus *Mycobacterium*." BMC Evol Biol 15: 21.

Kweon, O., S. J. Kim, R. D. Holland, H. Chen, D. W. Kim, Y. Gao, L. R. Yu, S. Baek, D. H. Baek, H. Ahn and C. E. Cerniglia (2011). "Polycyclic aromatic hydrocarbon metabolic network in *Mycobacterium vanbaalenii* PYR-1." J Bacteriol 193(17): 4326-4337.

Kweon, O., S. J. Kim, R. D. Holland, H. Chen, D. W. Kim, Y. Gao, L. R. Yu, S. Baek, D. H. Baek, H. Ahn and C. E. Cerniglia (2011). "Polycyclic aromatic hydrocarbon metabolic network in *Mycobacterium vanbaalenii* PYR-1." Journal of Bacteriology 193(17): 4326-4337.

Kweon, O., S. J. Kim, R. C. Jones, J. P. Freeman, M. D. Adjei, R. D. Edmondson and C. E. Cerniglia (2007). "A polyomic approach to elucidate the fluoranthene-degradative pathway in *Mycobacterium vanbaalenii* PYR-1." J Bacteriol 189(13): 4635-4647.

Larimer, F. W., P. Chain, L. Hauser, J. Lamerdin, S. Malfatti, L. Do, M. L. Land, D. A. Pelletier, J. T. Beatty, A. S. Lang, F. R. Tabita, J. L. Gibson, T. E. Hanson, C. Bobst, J. L. Torres, C. Peres, F. H. Harrison, J. Gibson and C. S. Harwood (2004). "Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*." Nat Biotechnol 22(1): 55-61.

Leahy, J. G. and R. R. Colwell (1990). "Microbial degradation of hydrocarbons in the environment." Microbiol Rev 54(3): 305-315.

Leclercq, S., F. M. Mian, A. M. Stanisz, L. B. Bindels, E. Cambier, H. Ben-Amram, O. Koren, P. Forsythe and J. Bienenstock (2017). "Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior." Nat Commun 8: 15062.

Lee, K., J. W. Park and I. S. Ahn (2003). "Effect of additional carbon source on naphthalene biodegradation by *Pseudomonas putida* G7." J Hazard Mater 105(1-3): 157-167.

Lee, M. L., M. Novotny and K. D. Bartle (1976). "Gas chromatography/mass spectrometric and nuclear magnetic resonance spectrometric studies of carcinogenic polynuclear aromatic hydrocarbons in tobacco and marijuana smoke condensates." Anal Chem 48(2): 405-416.

Lees, M. J. and M. L. Whitelaw (1999). "Multiple roles of ligand in transforming the dioxin receptor to an active basic helix-loop-helix/PAS transcription factor complex with the nuclear protein Arnt." Mol Cell Biol 19(8): 5811-5822.

Leser, T. D. and L. Molbak (2009). "Better living through microbial action: the benefits of the mammalian gastrointestinal microbiota on the host." Environ Microbiol 11(9): 2194-2206.

Levin, W., R. L. Chang, A. W. Wood, D. R. Thakker, H. Yagi, D. M. Jerina and A. H. Conney (1986). "Tumorigenicity of optical isomers of the diastereomeric bay-region 3,4-diol-1,2-epoxides of benzo[*c*]phenanthrene in murine tumor models." Cancer Res 46(5): 2257-2261.

Ley, R. E. (2015). "The gene-microbe link." Nature 518(7540): S7.

Leyden, J. J., K. J. McGinley, O. H. Mills and A. M. Kligman (1975). "Age-related changes in the resident bacterial flora of the human face." J Invest Dermatol 65(4): 379-381.

Lin, Q., L. Xiao-Chen, Y. Bo, L. Na, S. Min, C. Gang, L. Hui, Z. Jie and L. Fa-Sheng (2016). "Quantitative analysis of 3-OH-B[*a*]P and (+)-anti-BPDE as biomarkers of B[*a*]P exposure in rats." Biomed Chromatogr 30(3): 474-483.

Lips, P. (2006). "Vitamin D physiology." Prog Biophys Mol Biol 92(1): 4-8.

Lloyd-Price, J., G. Abu-Ali and C. Huttenhower (2016). "The healthy human microbiome." Genome Med 8(1): 51.

Lloyd, D. R. and P. C. Hanawalt (2000). "p53-dependent global genomic repair of benzo[*a*]pyrene-7,8-diol-9,10-epoxide adducts in human cells." Cancer Res 60(3): 517-521.

Loewenthal, L. J. (1963). "Anatomy and histochemistry of skin L961." Dermatologica 126: 380-387.

Lorentzen, R. J. and P. O. Ts'o (1977). "Benzo[*a*]yrenedione/benzo[*a*]pyrenediol oxidation-reduction couples and the generation of reactive reduced molecular oxygen." Biochemistry 16(7): 1467-1473.

Lu, L., Y. Wu, L. Zuo, X. Luo and P. J. Large (2014). "Intestinal microbiome and digoxin inactivation: meal plan for digoxin users?" World J Microbiol Biotechnol 30(3): 791-799.

Luch, A. (2005). "Nature and nurture - lessons from chemical carcinogenesis." Nat Rev Cancer 5(2): 113-125.

Luch, A. (2009). "On the impact of the molecule structure in chemical carcinogenesis." EXS 99: 151-179.

Lüers, F. and T. E. M. ten Hulscher (1996). "Temperature effect on the partitioning of Polycyclic Aromatic Hydrocarbons between natural organic carbon and water." Chemosphere 33(4): 643-657.

Lynch, S. V. (2016). "Gut Microbiota and Allergic Disease. New Insights." Ann Am Thorac Soc 13 Suppl 1: S51-54.

Mackay, D. and D. Callcott (1998). Partitioning and Physical Chemical Properties of PAHs. PAHs and Related Compounds: Chemistry. A. H. Neilson. Berlin, Heidelberg, Springer Berlin Heidelberg: 325-345.

MacLeod, C. T. and A. J. Daugulis (2003). "Biodegradation of polycyclic aromatic hydrocarbons in a two-phase partitioning bioreactor in the presence of a bioavailable solvent." Appl Microbiol Biotechnol 62(2-3): 291-296.

Marczynski, B., B. Pesch, M. Wilhelm, B. Rossbach, R. Preuss, J. U. Hahn, S. Rabstein, M. Raulf-Heimsoth, A. Seidel, H. P. Rihs, A. Adams, M. Scherenberg, A. Erkes, B. Engelhardt, K. Straif, H. U. Kafferlein, J. Angerer and T. Bruning (2009). "Occupational exposure to polycyclic aromatic hydrocarbons and DNA damage by industry: a nationwide study in Germany." Arch Toxicol 83(10): 947-957.

Marples, R. R. (1982). "Sex, constancy, and skin bacteria." Arch Dermatol Res 272(3-4): 317-320.

Martin, C. R. and E. A. Mayer (2017). "Gut-Brain Axis and Behavior." Nestle Nutr Inst Workshop Ser 88: 45-53.

Maurice, C. F., H. J. Haiser and P. J. Turnbaugh (2013). "Xenobiotics shape the physiology and gene expression of the active human gut microbiome." Cell 152(1-2): 39-50.

Merchant, H. A., F. Liu, M. Orlu Gul and A. W. Basit (2016). "Age-mediated changes in the gastrointestinal tract." Int J Pharm 512(2): 382-395.

Meulenberg, R., H. H. Rijnaarts, H. J. Doddema and J. A. Field (1997). "Partially oxidized polycyclic aromatic hydrocarbons show an increased bioavailability and biodegradability." FEMS Microbiol Lett 152(1): 45-49.

Meyer, B. K., M. G. Pray-Grant, J. P. Vanden Heuvel and G. H. Perdew (1998). "Hepatitis B virus X-associated protein 2 is a subunit of the unliganded aryl hydrocarbon receptor core complex and exhibits transcriptional enhancer activity." Mol Cell Biol 18(2): 978-988.

Meynet, P., S. E. Hale, R. J. Davenport, G. Cornelissen, G. D. Breedveld and D. Werner (2012). "Effect of activated carbon amendment on bacterial community structure and functions in a PAH impacted urban soil." Environ Sci Technol 46(9): 5057-5066.

Mohn, W. W., K. Westerberg, W. R. Cullen and K. J. Reimer (1997). "Aerobic biodegradation of biphenyl and polychlorinated biphenyls by Arctic soil microorganisms." Appl Environ Microbiol 63(9): 3378-3384.

Montiel-Castro, A. J., R. M. Gonzalez-Cervantes, G. Bravo-Ruiseco and G. Pacheco-Lopez (2013). "The microbiota-gut-brain axis: neurobehavioral correlates, health and sociality." Front Integr Neurosci 7: 70.

Moody, J. D., J. P. Freeman and C. E. Cerniglia (2005). "Degradation of benz[*a*]anthracene by *Mycobacterium vanbaalenii* strain PYR-1." Biodegradation 16(6): 513-526.

Moody, J. D., J. P. Freeman, P. P. Fu and C. E. Cerniglia (2004). "Degradation of benzo[*a*]pyrene by *Mycobacterium vanbaalenii* PYR-1." Applied and Environmental Microbiology 70(1): 340-345.

Moody, J. D., J. P. Freeman, P. P. Fu and C. E. Cerniglia (2004). "Degradation of benzo[*a*]pyrene by *Mycobacterium vanbaalenii* PYR-1." Appl Environ Microbiol 70(1): 340-345.

Moore, B. G. and A. P. Harrison, Jr. (1965). "Benzo[*a*]pyrene uptake by bacteria and yeast." J Bacteriol 90(4): 989-1000.

Morillo, E., A. S. Romero, C. Maqueda, L. Madrid, F. Ajmone-Marsan, H. Grcman, C. M. Davidson, A. S. Hursthouse and J. Villaverde (2007). "Soil pollution by PAHs in urban soils: a comparison of three European cities." J Environ Monit 9(9): 1001-1008.

Mueller, J. G., P. J. Chapman and P. H. Pritchard (1989). "Action of a fluoranthene-utilizing bacterial community on polycyclic aromatic hydrocarbon components of creosote." Appl Environ Microbiol 55(12): 3085-3090.

Murray, I. A., A. D. Patterson and G. H. Perdew (2014). "Aryl hydrocarbon receptor ligands in cancer: friend and foe." Nat Rev Cancer 14(12): 801-814.

Nebert, D. W. and T. P. Dalton (2006). "The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis." Nat Rev Cancer 6(12): 947-960.

Nebert, D. W., A. Puga and V. Vasiliou (1993). "Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer, and signal transduction." Ann N Y Acad Sci 685: 624-640.

Nebert, D. W., A. L. Roe, M. Z. Dieter, W. A. Solis, Y. Yang and T. P. Dalton (2000). "Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis." Biochem Pharmacol 59(1): 65-85.

Nelson, W. G. and M. B. Kastan (1994). "DNA strand breaks: the DNA template alterations that trigger p53-dependent DNA damage response pathways." Mol Cell Biol 14(3): 1815-1823.

Nichols, R. G., N. E. Hume, P. B. Smith, J. M. Peters and A. D. Patterson (2016). "Omics Approaches To Probe Microbiota and Drug Metabolism Interactions." Chem Res Toxicol 29(12): 1987-1997.

Nicholson, J. K., E. Holmes, J. Kinross, R. Burcelin, G. Gibson, W. Jia and S. Pettersson (2012). "Host-gut microbiota metabolic interactions." Science 336(6086): 1262-1267.

Oda, Y., B. Star, L. A. Huisman, J. C. Gottschal and L. J. Forney (2003). "Biogeography of the purple nonsulfur bacterium *Rhodopseudomonas palustris*." Appl Environ Microbiol 69(9): 5186-5191.

Oesch, F., E. Fabian, K. Guth and R. Landsiedel (2014). "Xenobiotic-metabolizing enzymes in the skin of rat, mouse, pig, guinea pig, man, and in human skin models." Arch Toxicol 88(12): 2135-2190.

Ohtake, F., K. Takeyama, T. Matsumoto, H. Kitagawa, Y. Yamamoto, K. Nohara, C. Tohyama, A. Krust, J. Mimura, P. Chambon, J. Yanagisawa, Y. Fujii-Kuriyama and S. Kato (2003). "Modulation of oestrogen receptor signalling by association with the activated dioxin receptor." Nature 423(6939): 545-550.

Okuda, H., K. Ogura, A. Kato, H. Takubo and T. Watabe (1998). "A possible mechanism of eighteen patient deaths caused by interactions of sorivudine, a new antiviral drug, with oral 5-fluorouracil prodrugs." J Pharmacol Exp Ther 287(2): 791-799.

Palackal, N. T., M. E. Burczynski, R. G. Harvey and T. M. Penning (2001). "The ubiquitous aldehyde reductase (AKR1A1) oxidizes proximate carcinogen trans-dihydrodiols to *o*-quinones: potential role in polycyclic aromatic hydrocarbon activation." Biochemistry 40(36): 10901-10910.

Palmer, C., E. M. Bik, D. B. DiGiulio, D. A. Relman and P. O. Brown (2007). "Development of the human infant intestinal microbiota." PLoS Biol 5(7): e177.

Pant, D., G. Van Bogaert, L. Diels and K. Vanbroekhoven (2010). "A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production." Bioresource Technology 101(6): 1533-1543.

Paschke, M., C. Hutzler, J. Brinkmann, F. Henkler and A. Luch (2015). "Polycyclic Aromatic Hydrocarbons in Newspaper Inks: Migration, Metabolism, and Genotoxicity in Human Skin." Polycyclic Aromatic Compounds 35(1): 32-40.

Poland A. and J. C. Knutson (1982). "2,3,7,8-Tetrachlorodibenzo-p-Dioxin and Related Halogenated Aromatic Hydrocarbons: Examination of the Mechanism of Toxicity." Annual Review of Pharmacology and Toxicology 22(1): 517-554.

Peng, R. H., A. S. Xiong, Y. Xue, X. Y. Fu, F. Gao, W. Zhao, Y. S. Tian and Q. H. Yao (2008). "Microbial biodegradation of polyaromatic hydrocarbons." FEMS Microbiol Rev 32(6): 927-955.

Perdew, G. H. (1988). "Association of the Ah receptor with the 90-kDa heat shock protein." J Biol Chem 263(27): 13802-13805.

Pierantozzi, M., A. Pietroiusti, L. Brusa, S. Galati, A. Stefani, G. Lunardi, E. Fedele, G. Sancesario, G. Bernardi, A. Bergamaschi, A. Magrini, P. Stanzione and A. Galante (2006). "*Helicobacter pylori* eradication and l-dopa absorption in patients with PD and motor fluctuations." Neurology 66(12): 1824-1829.

Pinyakong, O., H. Habe and T. Omori (2003). "The unique aromatic catabolic genes in *sphingomonads* degrading polycyclic aromatic hydrocarbons (PAHs)." The Journal of General and Applied Microbiology 49(1): 1-19.

Platzek, T., C. Lang, G. Grohmann, U. S. Gi and W. Baltes (1999). "Formation of a carcinogenic aromatic amine from an azo dye by human skin bacteria *in vitro*." Hum Exp Toxicol 18(9): 552-559.

Pointing, S. B. (2001). "Feasibility of bioremediation by white-rot fungi." Appl Microbiol Biotechnol 57(1-2): 20-33.

Pollenz, R. S., S. E. Wilson and E. J. Dougherty (2006). "Role of endogenous XAP2 protein on the localization and nucleocytoplasmic shuttling of the endogenous mouse Ahb-1 receptor in the presence and absence of ligand." Mol Pharmacol 70(4): 1369-1379.

Potter, B. (1966). "The physiology of the skin." Annu Rev Physiol 28: 159-176.

Price, P. B. (1938). "The Bacteriology of Normal Skin; A New Quantitative Test Applied to a Study of the Bacterial Flora and the Disinfectant Action of Mechanical Cleansing." The Journal of Infectious Diseases 63(3): 301-318.

Probst, M. R., S. Reisz-Porszasz, R. V. Agbunag, M. S. Ong and O. Hankinson (1993). "Role of the aryl hydrocarbon receptor nuclear translocator protein in aryl hydrocarbon (dioxin) receptor action." Mol Pharmacol 44(3): 511-518.

Puga, A., C. R. Tomlinson and Y. Xia (2005). "Ah receptor signals cross-talk with multiple developmental pathways." Biochem Pharmacol 69(2): 199-207.

Qin, J., R. Li, J. Raes, M. Arumugam, K. S. Burgdorf, C. Manichanh, T. Nielsen, N. Pons, F. Levenez, T. Yamada, D. R. Mende, J. Li, J. Xu, S. Li, D. Li, J. Cao, B. Wang, H. Liang, H. Zheng, Y. Xie, J. Tap, P. Lepage, M. Bertalan, J. M. Batto, T. Hansen, D. Le Paslier, A. Linneberg, H. B. Nielsen, E. Pelletier, P. Renault, T. Sicheritz-Ponten, K. Turner, H. Zhu, C. Yu, M. Jian, Y. Zhou, Y. Li, X. Zhang, N. Qin, H. Yang, J. Wang, S. Brunak, J. Dore, F. Guarner, K. Kristiansen, O. Pedersen, J. Parkhill, J. Weissenbach, P. Bork and S. D. Ehrlich (2010). "A human gut microbial gene catalogue established by metagenomic sequencing." Nature 464(7285): 59-65.

Ramadoss, P. and G. H. Perdew (2005). "The transactivation domain of the Ah receptor is a key determinant of cellular localization and ligand-independent nucleocytoplasmic shuttling properties." Biochemistry 44(33): 11148-11159.

Ramesh, A., A. Archibong, D. Hood, Z. Guo and B. Loganathan (2011). “Global Environmental Distribution and Human Health Effects of Polycyclic Aromatic Hydrocarbons.” Global Contamination Trends of Persistent Organic Chemicals CRC Press: 97-126.

Rath, C. M. and P. C. Dorrestein (2012). "The bacterial chemical repertoire mediates metabolic exchange within gut microbiomes." Curr Opin Microbiol 15(2): 147-154.

Reyes, H., S. Reisz-Porszasz and O. Hankinson (1992). "Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor." Science 256(5060): 1193-1195.

Ribeiro, R. A., C. W. Wanderley, D. V. Wong, J. M. Mota, C. A. Leite, M. H. Souza, F. Q. Cunha and R. C. Lima-Junior (2016). "Irinotecan- and 5-fluorouracil-induced intestinal mucositis: insights into pathogenesis and therapeutic perspectives." Cancer Chemother Pharmacol 78(5): 881-893.

Richter, C. A., D. E. Tillitt and M. Hannink (2001). "Regulation of subcellular localization of the aryl hydrocarbon receptor (AhR)." Arch Biochem Biophys 389(2): 207-217.

Riiser, A. (2015). "The human microbiome, asthma, and allergy." Allergy Asthma Clin Immunol 11: 35.

Sarkany, I. and C. C. Gaylarde (1968). "Bacterial colonisation of the skin of the newborn." J Pathol Bacteriol 95(1): 115-122.

Scharschmidt, T. C. and M. A. Fischbach (2013). "What Lives On Our Skin: Ecology, Genomics and Therapeutic Opportunities Of the Skin Microbiome." Drug Discov Today Dis Mech 10(3-4).

Schlezinger, J. J., D. Liu, M. Farago, D. C. Seldin, K. Belguise, G. E. Sonenshein and D. H. Sherr (2006). "A role for the aryl hydrocarbon receptor in mammary gland tumorigenesis." Biol Chem 387(9): 1175-1187.

Schmitt, R., H. R. Langguth, W. Püttmann, H. P. Rohns, P. Eckert and J. Schubert (1996). "Biodegradation of aromatic hydrocarbons under anoxic conditions in a shallow sand and gravel aquifer of the Lower Rhine Valley, Germany." Organic Geochemistry 25(1): 41-50.

Schneider, J., R. Grosser, K. Jayasimhulu, W. Xue and D. Warshawsky (1996). "Degradation of Pyrene, Benz[*a*]anthracene, and Benzo[*a*]pyrene by *Mycobacterium sp.* Strain RJGII-135, Isolated from a Former Coal Gasification Site." Applied and Environmental Microbiology 62(4): 1491-1491.

Schommer, N. N. and R. L. Gallo (2013). "Structure and function of the human skin microbiome." Trends Microbiol 21(12): 660-668.

Schoop, V. M., N. Mirancea and N. E. Fusenig (1999). "Epidermal organization and differentiation of HaCaT keratinocytes in organotypic coculture with human dermal fibroblasts." J Invest Dermatol 112(3): 343-353.

Schröder, S. (2010). "PAK in Lebensmitteln." Nachrichten aus der Chemie 58(4): 468-469.

Sepetdjian, E., A. Shihadeh and N. A. Saliba (2008). "Measurement of 16 polycyclic aromatic hydrocarbons in narghile waterpipe tobacco smoke." Food Chem Toxicol 46(5): 1582-1590.

Shankar, V., M. Gouda, J. Moncivaiz, A. Gordon, N. V. Reo, L. Hussein and O. Paliy (2017). "Differences in Gut Metabolites and Microbial Composition and Functions between Egyptian and U.S. Children Are Consistent with Their Diets." mSystems 2(1).

Shimada, T. (2006). "Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons." Drug Metab Pharmacokinet 21(4): 257-276.

Shimada, T. and Y. Fujii-Kuriyama (2004). "Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1." Cancer Sci 95(1): 1-6.

Siddens, L. K., A. Larkin, S. K. Krueger, C. A. Bradfield, K. M. Waters, S. C. Tilton, C. B. Pereira, C. V. Lohr, V. M. Arlt, D. H. Phillips, D. E. Williams and W. M. Baird (2012). "Polycyclic aromatic hydrocarbons as skin carcinogens: comparison of benzo[*a*]pyrene, dibenzo[*def,p*]chrysene and three environmental mixtures in the FVB/N mouse." Toxicol Appl Pharmacol 264(3): 377-386.

Silva, I. S., C. Santos Eda, C. R. Menezes, A. F. Faria, E. Franciscon, M. Grossman and L. R. Durrant (2009). "Bioremediation of a polyaromatic hydrocarbon contaminated soil by native soil microbiota and bioaugmentation with isolated microbial consortia." Bioresour Technol 100(20): 4669-4675.

Sims, R. C. and M. R. Overcash (1983). “Fate of polynuclear aromatic compounds (PNAs) in soil-plant systems.” Residue Reviews: Residues of Pesticides and Other Contaminants in the Total Environment. F. A. Gunther. New York, NY, Springer New York: 1-68.

Somerville, D. A. (1969). "The normal flora of the skin in different age groups." Br J Dermatol 81(4): 248-258.

Sorg, O. (2014). "AhR signalling and dioxin toxicity." Toxicol Lett 230(2): 225-233.

Souza, T., D. Jennen, J. van Delft, M. van Herwijnen, S. Kyrtoupolos and J. Kleinjans (2016). "New insights into B[*a*]P-induced toxicity: role of major metabolites in transcriptomics and contribution to hepatocarcinogenesis." Arch Toxicol 90(6): 1449-1458.

Sowada, J., A. Schmalenberger, I. Ebner, A. Luch and T. Tralau (2014). "Degradation of benzo[*a*]pyrene by bacterial isolates from human skin." FEMS Microbiol Ecol 88(1): 129-139.

Staudinger, T., A. Pipal and B. Redl (2011). "Molecular analysis of the prevalent microbiota of human male and female forehead skin compared to forearm skin and the influence of make up." J Appl Microbiol 110(6): 1381-1389.

Steffen, K. T., A. Hatakka and M. Hofrichter (2003). "Degradation of benzo[*a*]pyrene by the litter-decomposing basidiomycete *Stropharia coronilla*: role of manganese peroxidase." Appl Environ Microbiol 69(7): 3957-3964.

Steliga, T., P. Jakubowicz and P. Kapusta (2012). "Changes in toxicity during *in situ* bioremediation of weathered drill wastes contaminated with petroleum hydrocarbons." Bioresour Technol 125: 1-10.

Stingley, R. L., A. A. Khan and C. E. Cerniglia (2004). "Molecular characterization of a phenanthrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1." Biochem Biophys Res Commun 322(1): 133-146.

Stolpmann, K., J. Brinkmann, S. Salzmann, D. Genkinger, E. Fritsche, C. Hutzler, H. Wajant, A. Luch and F. Henkler (2012). "Activation of the aryl hydrocarbon receptor sensitises human keratinocytes for CD95L- and TRAIL-induced apoptosis." Cell Death Dis 3: e388.

Stringfellow, W. T. and M. D. Aitken (1995). "Competitive metabolism of naphthalene, methylnaphthalenes, and fluorene by phenanthrene-degrading *pseudomonads*." Appl Environ Microbiol 61(1): 357-362.

Sulc, M., R. Indra, M. Moserova, H. H. Schmeiser, E. Frei, V. M. Arlt and M. Stiborova (2016). "The impact of individual cytochrome P450 enzymes on oxidative metabolism of benzo[*a*]pyrene in human livers." Environ Mol Mutagen 57(3): 229-235.

Swanson, H. I. (2004). "Cytochrome P450 expression in human keratinocytes: an aryl hydrocarbon receptor perspective." Chem Biol Interact 149(2-3): 69-79.

Szafranski, S. P., Z. L. Deng, J. Tomasch, M. Jarek, S. Bhuju, M. Rohde, H. Sztajer and I. Wagner-Dobler (2017). "Quorum sensing of *Streptococcus* mutans is activated by *Aggregatibacter actinomycetemcomitans* and by the periodontal microbiome." BMC Genomics 18(1): 238.

Tarnow, P., C. Hutzler, S. Grabiger, K. Schon, T. Tralau and A. Luch (2016). "Estrogenic Activity of Mineral Oil Aromatic Hydrocarbons Used in Printing Inks." PLoS One 11(1): e0147239.

The human microbiome project consortium (2012). "Structure, function and diversity of the healthy human microbiome." Nature 486(7402): 207-214.

Thompson, A. L., A. Monteagudo-Mera, M. B. Cadenas, M. L. Lampl and M. A. Azcarate-Peril (2015). "Milk- and solid-feeding practices and daycare attendance are associated with differences in bacterial diversity, predominant communities, and metabolic and immune function of the infant gut microbiome." Front Cell Infect Microbiol 5(3).

Tomasinsig, L. and M. Zanetti (2005). "The cathelicidins--structure, function and evolution." Curr Protein Pept Sci 6(1): 23-34.

Tralau, T., J. Sowada and A. Luch (2015). "Insights on the human microbiome and its xenobiotic metabolism: what is known about its effects on human physiology?" Expert Opin Drug Metab Toxicol 11(3): 411-425.

Tremaroli, V. and F. Backhed (2012). "Functional interactions between the gut microbiota and host metabolism." Nature 489(7415): 242-249.

Tsai, J. C., M. Kumar and J. G. Lin (2009). "Anaerobic biotransformation of fluorene and phenanthrene by sulfate-reducing bacteria and identification of biotransformation pathway." J Hazard Mater 164(2-3): 847-855.

Turnbaugh, P. J. and J. I. Gordon (2009). "The core gut microbiome, energy balance and obesity." J Physiol 587(Pt 17): 4153-4158.

VanRooij, J. G., E. Vinke, J. De Lange, P. L. Bruijnzeel, M. M. Bodelier-Bade, J. Noordhoek and F. J. Jongeneelen (1995). "Dermal absorption of polycyclic aromatic hydrocarbons in the blood-perfused pig ear." J Appl Toxicol 15(3): 193-200.

Vasiliou, V., A. Pappa and D. R. Petersen (2000). "Role of aldehyde dehydrogenases in endogenous and xenobiotic metabolism." Chem Biol Interact 129(1-2): 1-19.

Veldhoen, M., K. Hirota, A. M. Westendorf, J. Buer, L. Dumoutier, J. C. Renauld and B. Stockinger (2008). "The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins." Nature 453(7191): 106-109.

Vyas, B. R. M., S. Bakowski, V. Šašek and M. Matucha (1994). "Degradation of anthracene by selected white rot fungi." FEMS Microbiology Ecology 14(1): 65-70.

Wang, Y., V. Leng, V. Patel and K. S. Phillips (2017). "Injections through skin colonized with *Staphylococcus aureus* biofilm introduce contamination despite standard antimicrobial preparation procedures." Sci Rep 7: 45070.

Weissenfels, W. D., M. Beyer and J. Klein (1990). "Degradation of phenanthrene, fluorene and fluoranthene by pure bacterial cultures." Appl Microbiol Biotechnol 32(4): 479-484.

Williams, R. T. (1971). "The metabolism of certain drugs and food chemicals in man." Ann N Y Acad Sci 179: 141-154.

Willison, J. C. (2004). "Isolation and characterization of a novel *sphingomonad* capable of growth with chrysene as sole carbon and energy source." FEMS Microbiology Letters 241(2): 143-150.

Wilson, I. D. and J. K. Nicholson (2017). "Gut microbiome interactions with drug metabolism, efficacy, and toxicity." Transl Res 179: 204-222.

Xue, W. and D. Warshawsky (2005). "Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: A review." Toxicology and Applied Pharmacology 206(1): 73-93.

Yadav, J. S., H. Doddapaneni and V. Subramanian (2006). "P450ome of the white rot fungus *Phanerochaete chrysosporium*: structure, evolution and regulation of expression of genomic P450 clusters." Biochem Soc Trans 34(Pt 6): 1165-1169.

Yazdi, A. S., M. Rocken and K. Ghoreschi (2016). "Cutaneous immunology: basics and new concepts." Semin Immunopathol 38(1): 3-10.

Ye, D., M. A. Siddiqi, A. E. Maccubbin, S. Kumar and H. C. Sikka (1996). "Degradation of Polynuclear Aromatic Hydrocarbons by *Sphingomonas paucimobilis*." Environmental Science & Technology 30(1): 136-142.

Yen, K. M. and I. C. Gunsalus (1982). "Plasmid gene organization: naphthalene/salicylate oxidation." Proc Natl Acad Sci U S A 79(3): 874-878.

Yen, K. M. and I. C. Gunsalus (1985). "Regulation of naphthalene catabolic genes of plasmid NAH7." J Bacteriol 162(3): 1008-1013.

Yoo, D. H., I. S. Kim, T. K. Van Le, I. H. Jung, H. H. Yoo and D. H. Kim (2014). "Gut microbiota-mediated drug interactions between lovastatin and antibiotics." Drug Metab Dispos 42(9): 1508-1513.

Yuan, S. Y., L. C. Shiung and B. V. Chang (2002). "Biodegradation of polycyclic aromatic hydrocarbons by inoculated microorganisms in soil." Bull Environ Contam Toxicol 69(1): 66-73.

8. List of Publications

Sowada J, Lemoine L, Schön K, Hutzler C, Luch A, Tralau T.

Toxification of polycyclic aromatic hydrocarbons by commensal bacteria from human skin. Archives of Toxicology, **2017**

Tkachenko A, Henkler F, Brinkmann J, **Sowada J**, Genkinger D, Kern C, Tralau T, Luch A.

The Q-rich/PST domain of the AHR regulates both ligand-induced nuclear transport and nucleocytoplasmic shuttling.

Scientific Reports, **2016**

Tralau T, **Sowada J**, Luch A.

Insights on the human microbiome and its xenobiotic metabolism: what is known about its effects on human physiology?

Expert Opinion on Drug Metabolism & Toxicology, **2015**

Sowada J, Schmalenberger A, Ebner I, Luch A, Tralau T.

Degradation of benzo[*a*]pyrene by bacterial isolates from human skin.

FEMS Microbiology Ecology, **2014**

Award

Congress of the Deutsche Gesellschaft für Pharmakologie und Toxikologie (DGPT) 2015

Award for the best poster in the field of toxicology

„Degradation of benzo[a]pyrene on the human skin leads to the formation of cytotoxic and genotoxic metabolites“