2 Introduction

2.1 Proliferation and cell cycle control

Multicellular organisms require precise control of the cell cycle during development and growth to define the size and form of each tissue. Disruption of cell cycle control consequently leads to severe defects, such as carcinogenesis[90]. Transitions between the four phases of the cell cycle (Figure 2-1) are regulated by cyclin dependent kinase complexes (CDKCs) composed of a regulatory cyclin subunit and a catalytic subunit, the cyclin dependent kinase (CDK). For instance, the phosphorylation of the tumor suppressor protein Rb by CDK4 and 6 leads to its inactivation and to the release of E2Fs. Thus, crossing the restriction point (R), the cell cycle enters irreversibly into the S-phase. Differentiated cells leave the cell cycle by entering into the stationary, irrevocable G_0-phase.

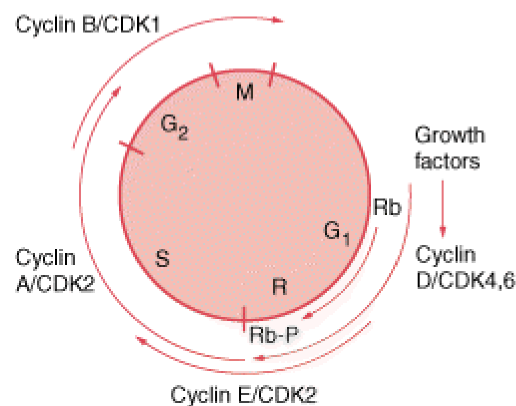

Figure 2-1: Regulation of the eukaryotic cell cycle.
Progression through the different cell cycle phases is regulated by timely regulated expression of G_1-phase (CyclinD/CDK4,6), S-phase (CyclinE/CDK2, CyclinA/CDK2) and mitotic (Cyclinb/CDK1) cyclin-dependent kinase complexes. At the restriction point (R) the Retinoblastoma protein (Rb) regulates the S-phase entry. This point is crossed only in a hyperphosphorylated condition (Rb-P)[47].

Another tumor suppressor protein involved in cell cycle regulation is p53. In case of DNA damage p53 blocks cell cycle progression through inhibition of specific CDKs via CDK inhibitor proteins (CIPs) at defined checkpoints in G_1 and G_2[64].

2.2 Apoptosis and Carcinogenesis

The term apoptosis describes a specific form of programmed cell death, in which locally and temporally confined signals initiate cell death. Apoptosis is executed by specific proteases, called ‘caspases’, as their active side contains a cysteine and their substrates are cleaved at an aspartic residue. Activation of caspases, subdivided into initiator and execution caspases, follows signal events that either take the intrinsic, or mitochondrial, pathway or the extrinsic signaling pathway, which involves the TNF receptor, with an optional link between both pathways. Execution of apoptosis is associated with membrane inversion, blebbing, fragmentation of the nucleus, chromatin condensation and DNA degradation. Finally, apoptotic bodies are cleared from the body by phagocytes. Apoptosis is induced when the cell homeostasis is disturbed or irreparable damage to DNA or cell organelles has occurred.

If the mechanisms to induce or execute apoptosis are disrupted one of the hallmarks of cancer is established[31]. These hallmarks, represented in Figure 2-2, were first described by Hanahan and Weinberg in 2000. According to this publication, a cell must undergo six alterations in cell physiology before becoming cancerous. These are: the acquisition of self-sufficiency in growth signals, an insensitivity to anti-growth signals, the above mentioned evasion of programmed cell death, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis.

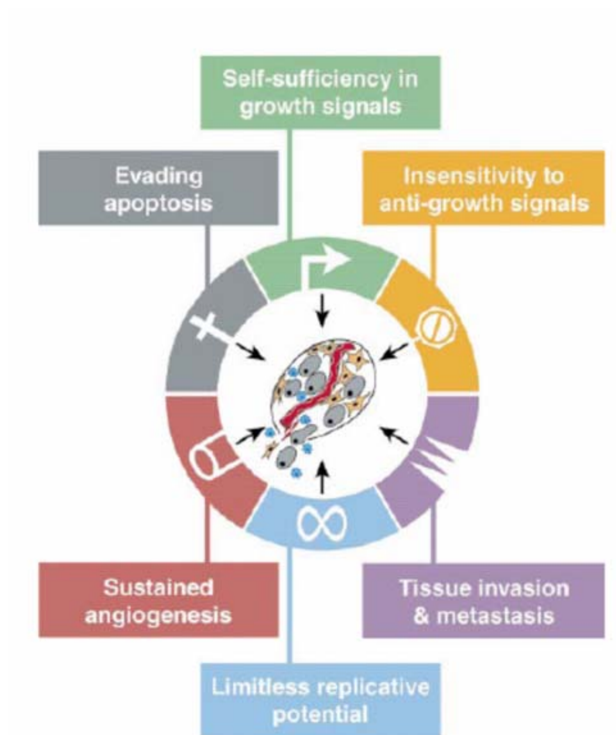

Figure 2-2: Acquired capabilities of cancer
To become cancerous, a cell must undergo all six hallmarks of a cancer cell, as displayed here. (Hanahan and Weinberg, 2000)

These changes collectively dictate malignant growth, with the consequence that loss of just one of these physiological alterations should lead to a block in cancer development or growth. The reintroduction of susceptibility to apoptotic stimuli, for instance, should prevent unlimited growth by killing the cell.

Limitless replicative potential is characterized by the loss of typical aging signs, such as a stop in proliferation through entering the G_0-cell cycle state or telomere shortening, which leads to a block in DNA replication. Together with self-sufficiency in growth signals, limitless replicative potential causes unrestrained proliferation of cancer cells. The Ras/Raf/MEK pathway is an example of a signaling pathway that renders cells self-sufficient of external growth factors, when it is constitutively activated by mutations. This pathway transduces external signals to the interior of the cell and regulates a broad spectrum of cellular activities, including proliferation, differentiation and cell survival [13; 45; 67] (Figure 2-3).

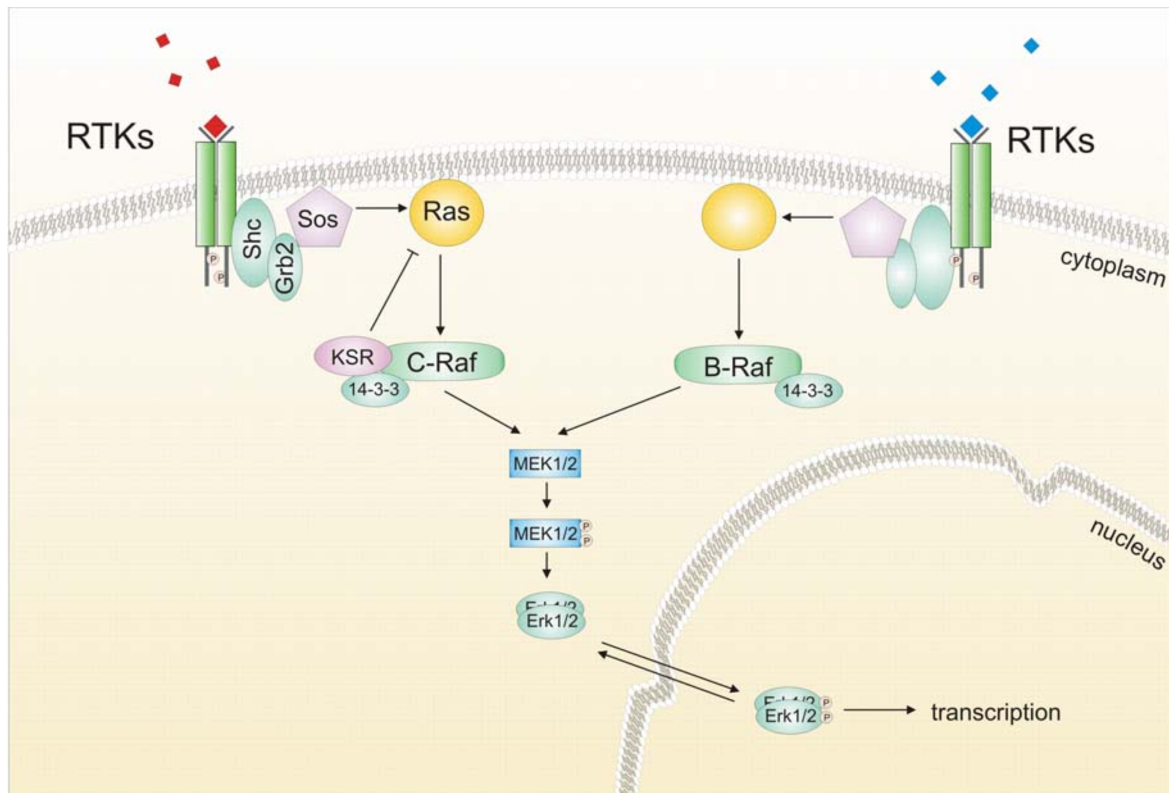

Figure 2-3: Growth factor signaling
A signaling pathway commonly disrupted or permanently active in cancer cells is the epidermal growth factor (EGF) receptor signaling pathway. Binding of EGF ligand to its receptor activates its intrinsic tyrosin kinase activity, which in turn leads to activation of guanine nucleotide exchange factors (GEFs). These again activate Ras by exchanging GDP for GTP. Activation of the kinase C-Raf leads to its translocation to the caveoli in the plasma membrane and the phosphorylation of MEK1/2. Active, phosphorylated Erk translocates to the nucleus where it acts as a transcription factor.

Mutated and, consequently, permanently active Ras is found in 30% of all human cancers[5; 10]. Particularly, the ability of a tumor to metastasize seems to be dependent on Ras/Raf activation[20; 70; 91]. When becoming malignant, tumor cells detach from the parent tumor, migrate through the body and invade an environment alien to its origin tissue, where they again start to proliferate. At this point, the cancer cells invade the whole body, which makes a treatment with currently available drugs and therapies impossible.

2.3 Prohibitin proteins

Proteins that are highly conserved throughout evolution are considered to be crucial for maintaining the health of an organism. These include the ubiquitously and abundantly expressed prohibitins, originally identified and subsequently named as inhibitors of G_1/S-phase progression[53]. Highly conserved prohibitin homologues have been found in bacteria[44], yeast[54], *C.elegans*[36], *Drosophila*[24] and

mammals[83]. Thus far, two prohibitins are known with a sequence similarity of 60% and an identity of 47% (Figure 2-4).

```
                  (1) 1        10        20        30        40        50        60        70                89
PHB 2/Bap37       (1) MAQNLKDLAGRLPAGPRGMGTALKLLLGAGAVAYGVRESVFTVEGGHRAIFFNRIGGVQQDTILAEGLHFRIPWFQYPIIYDIRARPRK
PHB 1             (1) -------------MAAKVFESIGKFGLALAVAGGVVNSALYNVDAGHRAVIFDRFRGVQ-DIVVGEGTHFLIPWVQKPIIFDCRSRPRN
Consensus         (1)               A K   S  K  LA A  A  V  ALF VDAGHRAI F R  GVQ D ILAEG HF IPW Q PIIFD RARPR

                 (90) 90        100       110       120       130       140       150       160              178
PHB2 /Bap37      (90) ISSPTGSKDLQMVNISLRVLSRPNAQELPSMYQRLGLDYEERVLPSIVNEVLKSVVAKFNASQLITQRAQVSLLIRRELTERAKDFSLI
PHB 1            (76) VPVITGSKDLQNVNITLRILFRPVASQLPRIFTSIGEDYDERVLPSITTEILKSVVARFDAGELITQRELVSRQVSDDLTERAATFGLI
Consensus        (90) I   TGSKDLQ VNISLRIL RP A  LP IF  IG DYDERVLPSI  EILKSVVAKF A  LITQR  VS  I  DLTERA  F LI

                (179) 179       190       200       210       220       230       240       250              267
PHB 2/Bap37     (179) LDDVAITELSFSREYTAAVEAKQVAQQEAQRAQFLVEKAKQEQRQKIVQAEGEAEAAKMLGEALSKN-PGYIKLRKIRAAQNISKTIAT
PHB 1           (165) LDDVSLTHLTFGKEFTEAVEAKQVAQQEAERARFVVEKAEQQKKAAIISAEGDSKAAELIANSLATAGDGLIELRKLEAAEDIAYQLSR
Consensus       (179) LDDVAIT LSF KEFT AVEAKQVAQQEA RA FLVEKA Q  K  II AEGDA AA LIA ALA    G I LRKI AA  IA  IA

                (268) 268         280       290       300
PHB 2/Bap37     (267) SQNRIYLTADNLVLNLQDESFTRGSDSLIKGKK
PHB 1           (254) SRNITYLPAGQSVLLQLPQ--------------
Consensus       (268) S N  YL A N VL
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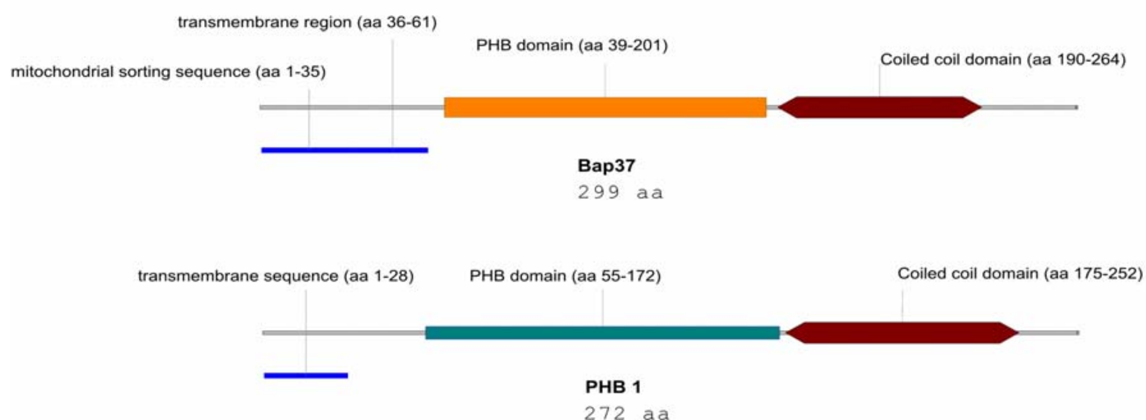

Figure 2-4: Consensus sequence of prohibitin proteins and their putative domains
Prohibitin 1 and 2 (Bap37) share a sequence similarity of 60%. Both proteins feature an unconventional non-cleavable N-terminal mitochondrial import signal. The PHB domain, taking up the main part of the protein structure, is the domain shared by all family members. The predicted C-terminal coiled coil domain is assumed to link both proteins.

While most research focused on prohibitin 1, recent studies show that prohibitin 2 plays a likewise important role in maintaining the homeostasis of the cell[43]. Although research interest to date is high no clear role has been ascertained for prohibitins. They are considered to be involved in regulation of the cell cycle[42; 89] apoptosis[28; 29], senescence[18; 19; 51], as well as embryonic development[24], and are chaperones to mitochondrial proteins[79; 82]. While overexpressed but not mutated prohibitin 1 is found in a relevant percentage of cancers[3], deletion of Cc, the prohibitin 1 homolog in *Drosophila*, leads to a lethal growth arrest at the larval stage[24].

Prohibitin 2 has multiple functions in different tissues and organelles, which is reflected in the number of names used to describe it. A common term is prohibitone[4; 48], as a repressor of estrogen receptor activity it is called REA[58], and

because of its interaction with IgM in the membrane of B-cells it was named BAP37 (B-cell activating protein of 37 kDa)[83].

Cell fractionation of rat liver showed almost all of the prohibitin proteins to be localized in mitochondria, with some protein in the lysosomal and ribosomal fractions and none in the cytoplasm. Observed nuclear localization was traced back to contaminations during preparation[55]. Nevertheless Chellappan *et al.* appear to have identified a nuclear localization for prohibitin 1 in two breast cancer cell lines - MCF-7 and T47D cells. Upon camptothecin induced apoptosis prohibitin 1 translocates from the nucleus to the mitochondria[29]. Prohibitin 2 is likewise mainly located to the mitochondria with traces of overexpressed protein found in the nucleus upon induction with estrogen[43]. Interestingly, prohibitins carry out a self stabilizing role on protein level. Loss of one protein by RNA interference approaches or by a knockout in yeast, leads to the reduction of the other on protein level only[8; 43; 79]

2.4 Prohibitin 1 as a tumor suppressor

The human prohibitin 1 gene is a candidate tumor suppressor locus that maps to a region of chromosome 17 (17q21) commonly deleted or mutated in breast tumors[73; 92]. Research on prohibitin started out in the early nineties with the discovery of its anti-proliferating function when microinjected into fibroblasts [63]. This attribute was later ascribed to its 3'UTR[42], thus assigning it to the novel class of non coding RNAs.

The importance of the 3'UTR for regulating proliferation was demonstrated when point mutations in this region where found in a relevant number of cancers[41]. According to Jupe and colleagues[42; 52] the prohibitin T allele, a single nucleotide polymorphism (SNP) originally described as the point mutation C->T at position 729bp, creates a variant that lacks this antiproliferative activity. However, numerous studies contradicted these findings, showing no relevance of prohibitin polymorphisms to breast cancer risks[12; 76; 77].

At the same time, the protein itself is still the subject of intensive research, which attempts to clarify its function as a tumor suppressor. Wang *et al.* report that prohibitin 1 interacts with pRb and regulates E2F function[88; 89]. Consequently, the obtained block on E2F function as transcription factor inhibits cell cycle progression.

2.5 Prohibitins in apoptosis

As stated in Chapter 2.3, prohibitin 1 translocates from the nucleus to the mitochondria upon apoptosis induction with camptothecin, a topoisomerase I inhibitor[28]. This and the fact that prohibitins are localized to the mitochondria, an organelle frequently involved in mediating and enhancing apoptotic stimuli, make prohibitins the target of apoptotic research. If overexpressed, prohibitin 1 protects the cell from camptothecin and growth factor withdrawal induced apoptosis[29; 86]. This result is consistent with observations that siRNA-mediated knockdown of prohibitin 1 sensitizes HeLa cells to apoptosis induced by cisplatin, a DNA crosslinker[69]. Furthermore, due to protein stabilization, prohibitin 2 protects Hax 1 a mitochondrial anti-apoptotic protein from degradation[43]. Consequently, loss of prohibitin 2 leads to apoptosis. A loss of Hax 1 with reduced prohibitin 1 protein levels was not observed, although loss of prohibitin proteins only occurs with a double knockdown. Protein stabilization between Hax 1 and prohibitin 2 should also be absent in case of a prohibitin 1 knockdown.

2.6 Prohibitins in Senescence

Although the roles of prohibitins in carcinogenesis and apoptosis have been discussed separately, they are in fact tightly connected. Alterations in the cell resulting in an evasion of apoptosis establish one of the hallmarks of carcinogenesis (see Chapter 2.2). The same applies to the role of prohibitins in senescence. Replicative senescence is a status in a cell in which proliferation stops irreversibly but metabolic function is maintained. This can be caused by oxidative stress, overexpression of oncogenes like Ras and Raf or as a response to DNA damage, including the shortening of telomeres below a certain threshold[15; 57; 74]. Senescence is attained through a stop in G_1-cell cycle phase. Combined with a loss of membrane potential in mitochondria, this leads to an overall degradation of the cell. Senescence, therefore, provides a potential mechanism to suppress tumor development[14]. If a cell escapes the restraints of replicative senescence, it becomes ‘immortal’, a phenotype often seen in cancer cells.

Prohibitin mRNA was shown to inhibit progress from the G_1 phase into the S phase of the cell cycle[53]. During development and in young cells, two isoforms of prohibitin can be detected of which the smaller is phosphorylated or modified in other ways. As this smaller, modified form is lost in older cells[51; 53; 84], this condition

is reminiscent of the loss of phosphorylated Rb in senescent cells[80]. It was likewise observed that in senescent cells prohibitin expression was decreased by transcriptional inactivation[19; 68] . While the previous observations were made in human fibroblasts, studies in yeast affirm the idea of prohibitin being involved in senescence. In yeast, a deletion of either prohibitin 1 (Phb1p) or prohibitin 2 (Phb2p) leads to a decreased replicative lifespan[18]. Surprisingly, since a single gene deletion automatically leads to a double knockout, double deletion of Phb1p and Phb2p results in an even greater life span decrease than the loss of either prohibitin gene alone[68].

Further analysis revealed that the cause of this aging phenotype might be a defect in the segregation of mitochondria from old mother cells, which have budded for several generations, to their daughter cells. Here, old yeast cells display pronounced defects in the mitochondrial morphology, which become shorter and less tubular[68].

The importance of prohibitin 1 as an inhibitor of apoptosis and its putative role as a tumor suppressor through regulation of E2F mediated transcription appear quite contradictory, considering the hallmarks of cancer, proposed by Hanahan and Weinberg (Chapter 2.2). Given that reduced prohibitin 1 levels could be used as a marker of senescence, the idea that prohibitin 1 acts as a negative regulator of the cell cycle is therefore questionable. Furthermore, research describing the tumor suppressing function of prohibitin 1 was performed under overexpressed conditions, while disclosing its function as apoptosis inhibitor or its role in senescence employed knockdown/ knockout techniques.

2.7 Prohibitins function as mitochondrial chaperones

As already indicated by studying mitochondria segregation in prohibitin deletion mutants (Chapter 2.6), prohibitins perform a role as mitochondrial chaperones. Furthermore, mitochondria occupy the central focus in apoptotic signaling processes, therefore potentially providing the stage for the apoptotic function of prohibitins.

As for most mitochondrial proteins, such as members of the mitochondrial import machinery, research to identify the role of prohibitins in mitochondria was mainly performed in yeast. Both prohibitins contain an atypical import signal. Yeast Phb2p

is targeted to the mitochondria by a bipartite non-cleavable sequence located in the first 61 amino acids, whereas yeast Phb1p features an unconventional non-cleavable N-terminal presequence in its first 28 amino acids. Both proteins require the translocase of the inner mitochondrial membrane (Tim23 complex) for membrane insertion and stable accumulation in the inner membrane[82].

Coates *et al.* were the first to show that prohibitins form a complex in the mitochondrial inner membrane[59]. Multimeres, consisting of 16-20 prohibitin molecules and presumably linked by their C-terminal coiled coil domains, act as chaperones to stabilize mitochondrial proteins and facilitate respiratory complex assembly[61; 79]. Additional work by Langer *et al.* showed that this complex was circularly arranged around the m-AAA protease (Figure 2-5).

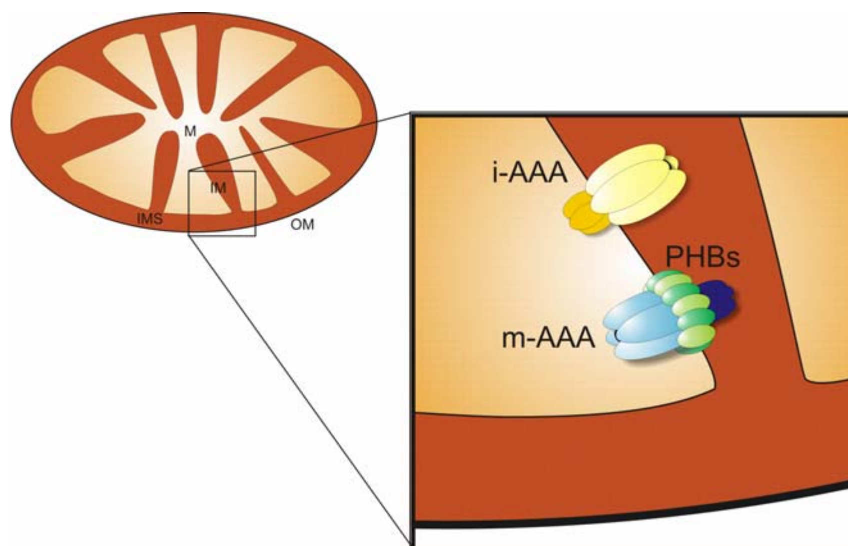

Figure 2-5: Prohibitin/m-AAA complex in the inner mitochondrial membrane
Prohibitin (PHB) proteins (1 and 2) form a complex with the m-AAA (ATP-associated with diverse cellular activity) protease in the inner mitochondrial membrane (IM). Whereas the i-AAA protease reaches with its catalytic side into the inter-membrane space (IMS), the m-AAA protease acts towards the matrix (M) Electronmicroscopy revealed that 16-20 prohibitin molecules form a ring like structure, 260Å in diameter. OM-outer membrane.

2.7.1 m-AAA protease

The AAA proteases are ATP dependent proteases (ATP-associated with diverse cellular activity) of the inner mitochondrial membrane. Their proteolytic domains have metal-dependent peptidase activity[49]. While the active site of the i-AAA protease reaches into the inter-membrane space, m-AAA exposes its catalytic site towards the matrix. In yeast, on which most of the studies were done, the m-AAA protease consists of two subunits, Yta10p and Yta12p, and regulates the turnover of non-assembled inner membrane proteins[2,79]. Their homologues in vertebrates are paraplegin and AFG3L2, respectively.

Prohibitins form an alternating high molecular weight ring complex around the m-AAA protease with a total molecular weight of 1.2 MDa and the outer bounds of 270x200 Å[79]. This complex stabilizes newly synthesized mitochondrial translation products[82]. Among the target proteins are cytochrome *c* oxidase (Cox1p) and cytochrome *b* (Cobp), two proteins transcribed from mitochondrial genes with introns. While the deletion of one of the m-AAA subunits leads to an impaired degradation of non-assembled inner membrane proteins and an impaired assembly of respiratory chain complexes[2], the deletion of either Phb1p or Phb2p leads to an accelerated turnover of these proteins[79]. A deletion of Phb1p or Phb2p in the background of either ΔYta10p or ΔYta12p impairs cell growth, implying a connection between the m-AAA protease and prohibitins.

2.7.2 OPA1 and PARL

Mitochondria are dynamic organelles, frequently undergoing marked remodeling and thought to be regulated by a balance between fusion and fission events. Often during the life cycle of a cell (e.g. during cell division), mitochondria need to divide and fuse. In addition, one of the hallmarks of apoptosis is the fragmentation of mitochondria. These alterations in the mitochondrial network are regulated by the mitochondrial fission (Drp1, Fis1) and fusion (OPA1, Mfn1 and Mfn2) proteins.

OPA1 is a dynamin related GTPase located in the inter-membrane space (IMS) but is tightly associated with the inner membrane[66]. It is transcribed in eight splice variants and cleaved into several active forms[21]. Due to these many modifications, the protein expression pattern and function of OPA1 is difficult to determine. Recent work reveals the importance of m-AAA for correct cleavage of OPA1. The m-AAA subunit paraplegin is necessary for OPA1s maturation and the expression of its two endogenous large fragments and three small fragments, which are visible in western blots. However, it is hitherto unclear if m-AAA cleaves OPA1 directly, or whether it cleaves its assigned protease, PARL (presenilin-associated rhomboid-like), thereby activating it and enabling the processing of OPA1 [17; 34].

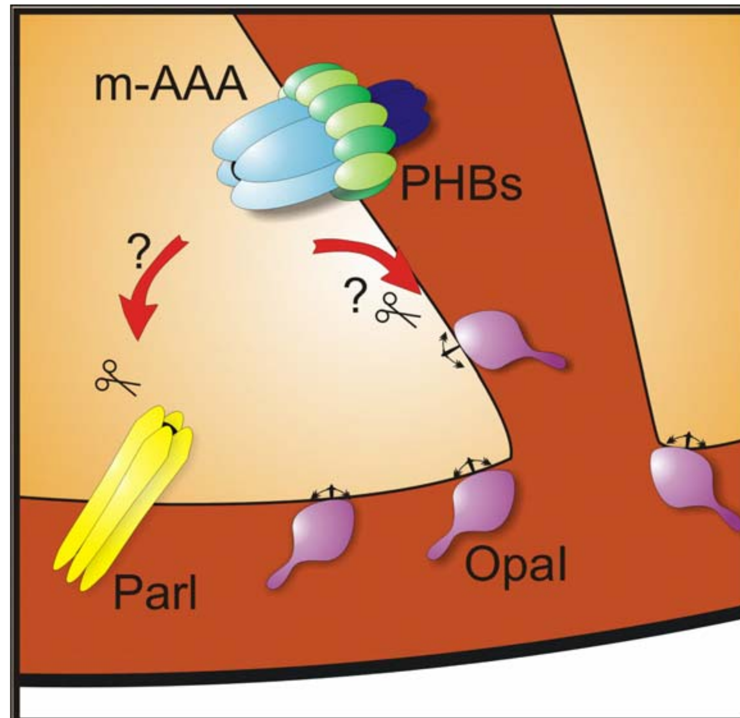

Figure 2-6: Processing of OPA1 is m-AAA dependent
The m-AAA protease is required for the maturation of respiratory chain complex proteins. It was shown as well, that processing of OPA1 requires m-AAA protease but it is not clear yet whether m-AAA directly cleaves OPA1 or if the activation of the protease PARL by m-AAA is the first step in OPA1 maturation.

PARL is a serin protease, located in the inner mitochondrial membrane. Studies in yeast showed, that this protease is necessary for the processing of cytochrome *c* peroxidase (Ccp1p) and Mgm1p, the ortholog to human OPA1. Loss of PARL leads to mitochondria fragmentation and reduced cell growth[56]. In contrast to lower eukaryotes, vertebrate PARL exhibits a specific cleavage site in the Pβ-domain and features three phosphorylation sites (S^{65},T^{69},S^{70})[75]. Moreover it was shown that cleavage of the Pβ-fragment occurs only if these residues are dephosphorylated, and that this cleavage consecutively initiates mitochondria fragmentation[37]. Scorrano *et al.* showed that, in vertebrates, PARL generates an antiapoptotic fragment by cleaving OPA1. This fragment, representing 4% of total OPA1 levels, showed no fusion activity. Instead it was freely located in the IMS closing the cristae through homodimerisation with full length OPA1, thereby keeping cytochrome c isolated in the cristae and preventing its early release in the IMS upon an apoptotic stimulus[27] (Figure 2-7).

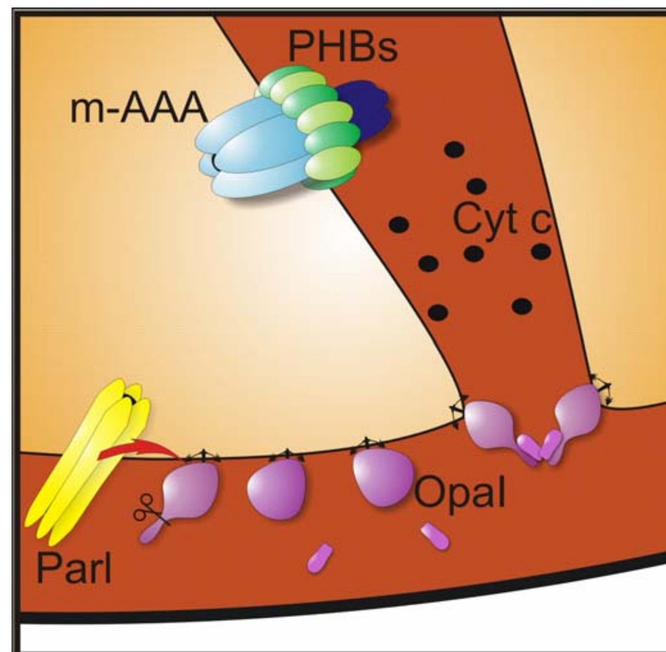

Figure 2-7: OPA1 requires PARL and m-AAA
for processing and maturation, respectively. The cleavage of the fusion protein OPA1 by PARL generates a small fragment in the IMS that through forming homo-oligomeres with full length OPA1, closes the cristae, preventing an early release of cytochrome *c*. This antiapoptotic property of OPA1 is additional to its function as mitochondrial fusion protein[27].

Understanding the role of prohibitins in maintaining mitochondrial homeostasis is impeded by confining research to common methods of analysis. The use of yeast cells to identify the function of mitochondrial proteins is popular due to its simplicity. However, the results obtained in yeast studies are not universally applicable, as their mitochondrial structure is slightly different to that of vertebrates and some of their proteins associated with the respiratory chain complexes, like Ccp1p, do not have vertebrate homologues[46; 56; 75].

Prohibitins were shown to be mitochondrial chaperones, associated with the m-AAA protease but further functions are possible. An effect on the maturation of mitochondrial fission or fusion proteins is for instance probable, as a loss of prohibitin expression leads to mitochondrial fragmentation[43].

2.8 Cell Migration

The migration of cells is a directed process. During the course of *chemotaxis*, cells migrate towards or along a gradient of diffusible chemicals like cytokines or growth factors. Initial cell polarization is a prerequisite for cell migration as well as for oriented cell division in tissues[23]. This polarity can be established through receptor signaling.

The receptor-ligand interaction leads to local protrusions, facilitated by actin polymerization near the receptor. Consequently a polarity gradient is established within the cell. Activation of RacGTPases through receptor signaling leads to further actin polymerization and protrusion formation which is visible through the extension of lamellipodia (Figure 2-8).

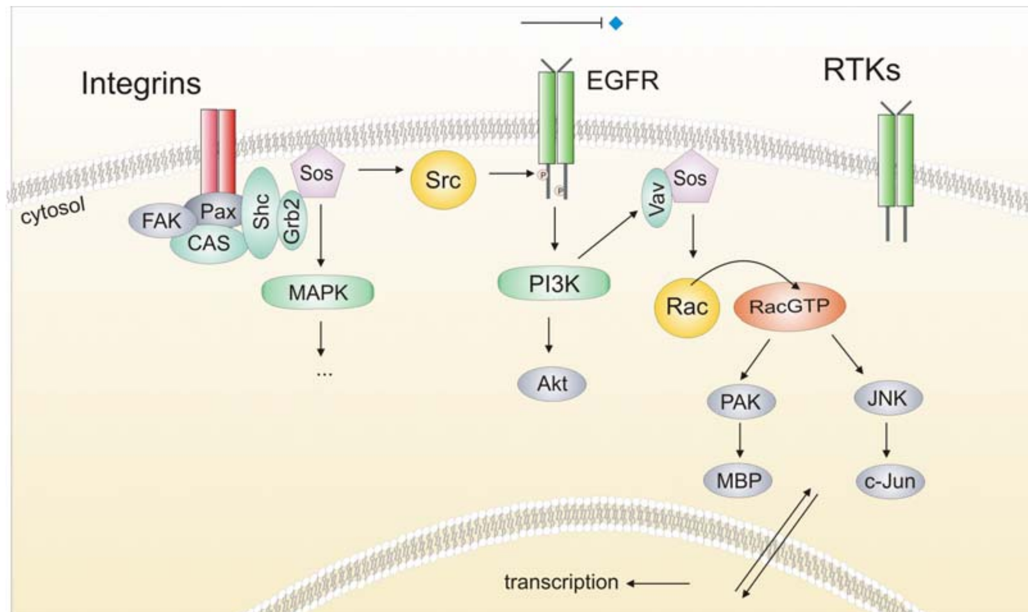

Figure 2-8: Growth factor signaling in migration
Activation of growth factor signaling is the first step in induction of cell polarization. This can be promoted by integrin mediated signal transduction in growth factor depleted conditions. Here, through cell-matrix contacts via integrins, receptor tyrosin kinases (RTKs) are activated, consequently leading to actin polymerization and the activation of small GTPases like Rac. Under growth conditions, direct interaction of the RTK ligand with its receptor leads to migration.

Microtubules consist of tubulin bundles and together with actin filaments induce polarity. Attached to the microtubule organizing center (MTOC) or centrosome, they present a positive (barbed) and negative (pointed) end with the positive end reaching into the protrusive region of the cell. Protrusive actin polymerization leads to a reorientation of the centrosomes and reinforces the extracellular polarity information that the actin cytoskeleton receives in a positive feedback loop (Figure 2-9).

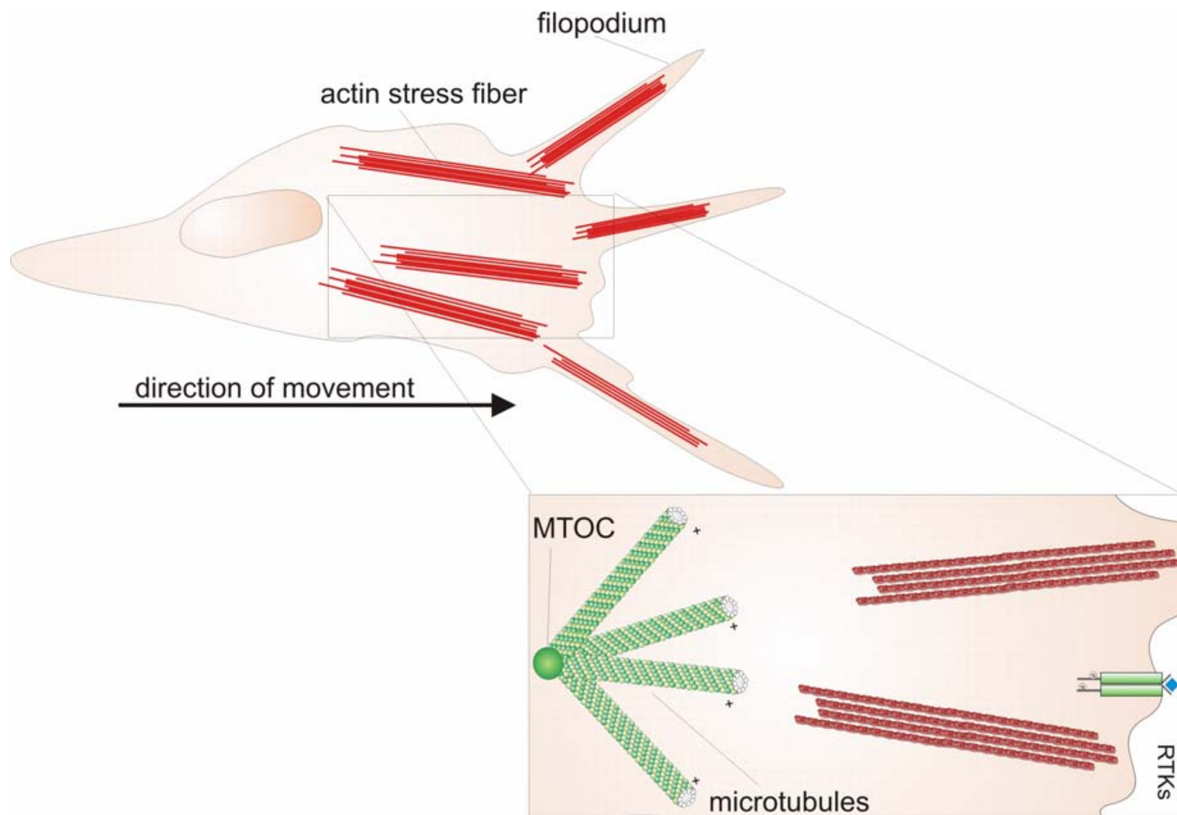

Figure 2-9: Protrusion formation upon activation of growth factor receptors
Initialization of a gradient is the first step towards cell migration. One sided activation of growth factor receptors through ligand binding or cell adhesion facilitates actin polymerization near the receptor. Formation of stress fibers is followed by protrusion formation and the extension of lamellipodia. Likewise involved in reinforcing polarity are microtubles, attached to the microtubule organizing center, or centromere

Polarized cells move on the extracellular matrix (ECM) or, more specifically, through the degradation of ECM proteins, *via* secreted matrix-metalloproteinases. ECM proteins are typically large glycoproteins, including fibronectins, collagens and laminins. This degradation therefore clears the path through the matrix, can promote cell detachment so that the cell can move onward or it can release extracellular signal proteins that stimulate cell migration[1].

Since cells are embedded in tissues through cell-cell contact or cell-matrix attachment, these interactions need to be abrogated before migration can take place. Four different cell-cell contacts are described (Figure 2-10):

Tight junctions seal neighboring cells together in an epithelial sheet to prevent leakage of molecules between them. An *adherens junction* joins an actin bundle in one cell to a similar bundle in a neighboring cell while *desmosomes* join the intermediate filaments in one cell to those in a neighbor. A g*ap junction* allows the passage of small water-soluble ions and molecules between cells.

Cell-matrix attachment is observed through *hemidesmosomes*. They anchor intermediate filaments in a cell to the basal lamina. *Focal adhesions* bind cells to the extra cellular matrix.

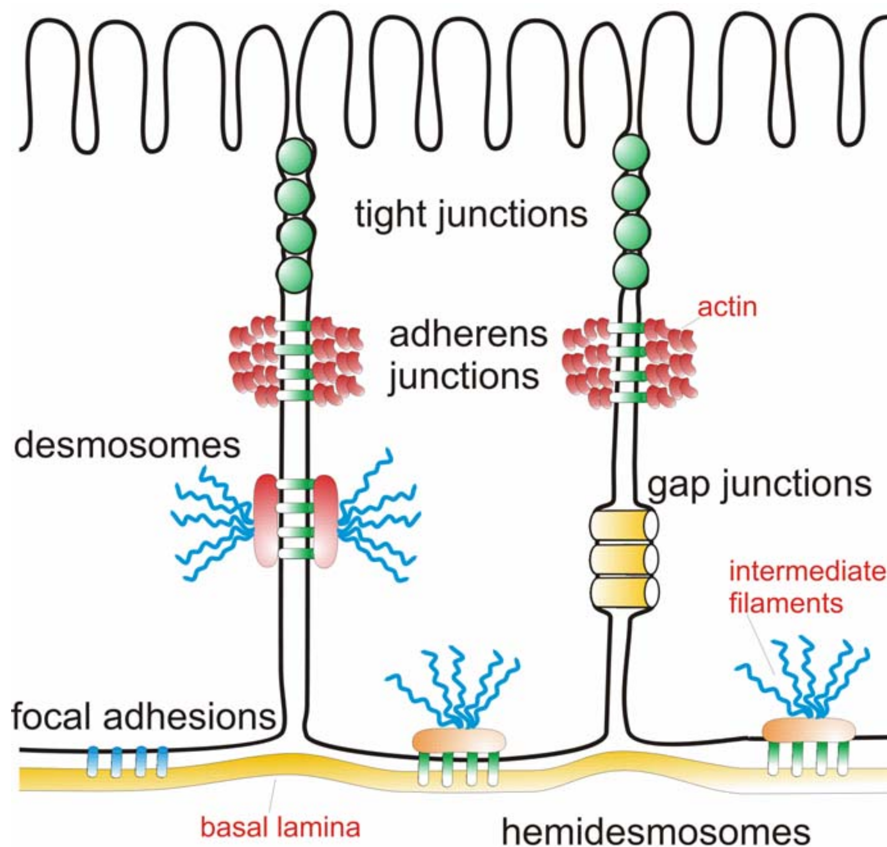

Figure 2-10: Cell-cell contacts and cell matrix contacts
(Drawing based on epithelial cells of the small intestine, Alberts *et al.* 4th ed.[1])

The cell-cell contacts are mediated by transmembrane adhesion proteins from the cadherin family like N-, E- and P-cadherin while cell-matrix contacts comprise integrins as transmembrane adhesion proteins. Integrin receptors are composed of two subunits, α- and β-integrin, each subdivided in 19 α- and 8 β- subunits, respectively (Figure 2-11).

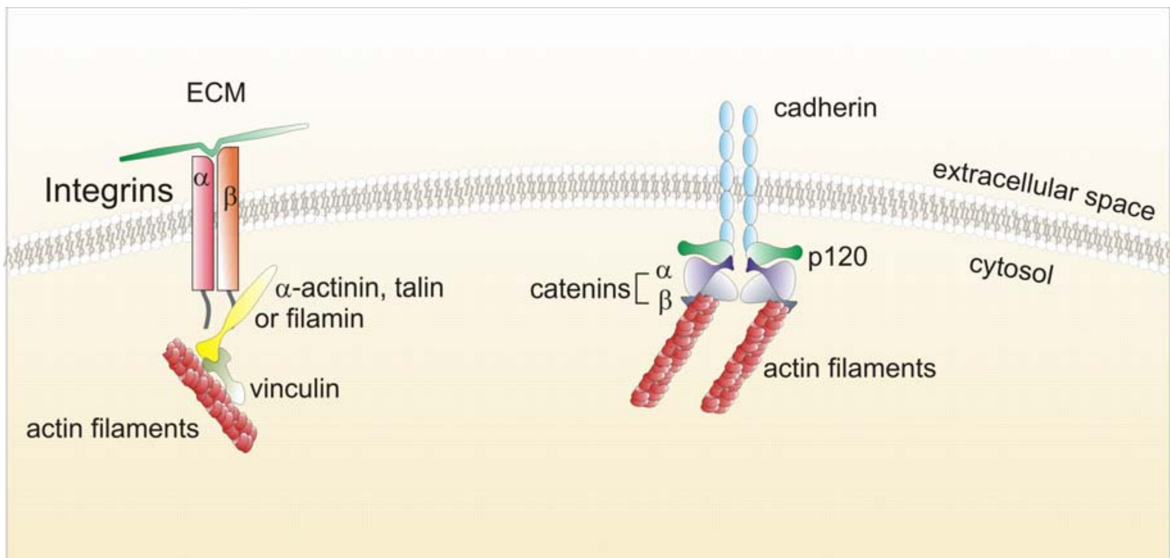

Figure 2-11: Signaling in cell-cell and cell-matrix contacts
Cell are attached to their surroundings via transmembrane proteins. Integrins mediate cell-matrix contacts, while cadherins anchor cells to each other. When activated, both contacts mediators lead to a polymerization of actin and induce cell polarity *via* local receptor activation.

It may seem unlikely that prohibitins play a role in cell migration. Nevertheless, an siRNA-mediated knockdown of prohibitin leads to a clumping phenotype in HeLa cells, comparable to that of a Her2/ErbB2 or Rac knockdown via RNA interference. The cells in clumps show marked membrane staining for pan-cadherin and β-catenin. Addition of PMA (phorbol ester) dissolves the clumps[69].

3 Objectives

Prohibitins are highly conserved proteins, maintaining cell homeostasis in all organisms from yeast to man. Areas of prohibitin research are numerous, comprising fields as diverse as steroid hormone signaling, cell cycle regulation and mitochondrial chaperoning. Prohibitin 1s mRNA is considered to have a regulatory function as a tumor suppressor and its protein was shown to interact with E2F, thereby extending the tumor suppressing function to the protein level. However, given that prohibitin knockouts are seemingly lethal and that a loss of prohibitins in yeast leads to a reduced replicative live span, the role of prohibitin 1 as a tumor suppressor is debated.

As the emergence of siRNA technology allows the extention of prohibitin research to loss of function studies, the first aim of the present study is to determine if prohibitin functions as a tumor suppressor. As the field of prohibitin research is wide spread, the fulfillment of this aim would help organize the abundance of sometimes controversial information.

The second aim of this study is to characterize the function of prohibitin 1 and prohibitin 2 as mitochondrial chaperones. Until now, yeast has been the species of choice for the analysis of mitochondrial proteins, but increasing reports suggest that the yeast system does not equate directly to vertebrates. As an increased knowledge of the human system is the chief aim of prohibitin research, loss of function studies must be extended to mammalian cells.

Using shRNA mediated RNA interference in mammalian cells, a long term down-regulation of prohibitins can be achieved, thus enabling the analysis of mitochondrial function, structure and protein composition in a cell. This will lead to a clearer understanding of the role of prohibitins as mitochondrial chaperones.