1 INTRODUCTION

Everything we know about the outside world we experience through our five senses: sight, hearing, smell, taste and touch. Although sensory perception differs in detail there are a number of common biological criteria underlying this process.

First of all, when a physical stimulus impinges onto specialised receptors in the periphery of the body it is transduced into trains of nerve impulses, which are interpreted by the brain. Subsequently we perceive a typical sensation evoked by specific physical stimuli. In addition to the conscious experience of the external world, we also receive sensory information from within the body that usually does not reach consciousness like regulation of body temperature, blood pressure, reflex and involuntary movements. Such senses are essential for controlling autonomic body functions or movement and motility. Secondly, although sensory receptors have different morphologies and organisation they extract the same elementary information from a given stimulus: modality, intensity, duration and location. Each receptor is sensitive to a limited range of physical stimuli. Different sense organs produce distinctive subjective sensation, but sensation evoked by a stimulus depends on the type of the sense organ that has been stimulated rather than on the nature of the stimulus. However, different sense organs are grouped accordingly to the kind of energy that they are most sensitive to, namely chemical, thermal, electrical (including electromagnetic) and mechanical energy.

Thirdly, sensory systems are topographically organised. Except for the olfactory system transmitting sensory information directly to the cerebral cortex, the thalamus is the essential relay point in processing sensory information. The thalamic neurones project to a specific primary sensory area of the cerebral cortex where conscious perception takes place.

Of Aristotle's five senses we have a well-founded knowledge about the molecular mechanisms involved in the transduction of sight, smell and taste (see also Fain, G.L. Sensory Transduction, Sinauer Associates Inc. 2003). The study is concentrated on

cutaneous touch sensation in mammals and its molecular basis, which is largely not understood.

1.1 Sensory neurone mechanotransduction

More or less all creatures respond to mechanical stimulation. At the single cell level mechanical forces or pressure applied to the cell membrane are important for cell volume regulation or for prevention of poly-sperm fertilisation for instance. Considering a more complex organism many other biological processes, such as blood pressure regulation, proprioreception, balance as well as touch and hearing are based upon the transduction of mechanical stimuli into electrical signals, a process that is called mechanotransduction (for further reading see also Fain, G.L. Sensory Transduction, Sinauer Associates Inc. 2003). The diversity of biological processes in which mechanical signals are converted into cellular responses makes the understanding of the underlying mechanisms important.

In sensory neurone mechanotransduction receptive afferent endings of specialised sensory neurones receive a mechanical stimulus that causes a physical deformation or deflection in these endings. Subsequently mechanosensitive ion channels located exclusively in the cell membrane of the afferent terminals open, a cation influx leads to depolarisation of the cell membrane and a local receptor potential is generated (reviewed by (Catton, 1970; Gillespie and Walker, 2001; Hu et al., 2006) (French, 1992)). If the receptor potential breaks a certain threshold, action potentials are initiated within milliseconds (Loewenstein, 1959) and are propagated along the axon to the central nervous system (CNS). One reason for the difficulties in exploring those molecules is that mechanoreceptor endings are very fine structures often embedded in specialised surrounding end organs, which makes them very difficult to examine *in vivo* (Garcia-Anoveros and Corey, 1997). In addition, mechanosensitive ion channels are often very sparse. In this respect, and also due to the fact that chemicals that interact with putative mechanotransducers with high affinity and high specificity are not yet known, it is also not a trivial task to get access to the channels or to purify those channel proteins.

In recent years progress in understanding sensory neurone mechanotransduction has resulted from an assembly of research combining genetic, genomic and electrophysiological approaches. Candidate molecules include ion channel subunits of the transient receptor potential (TRP) superfamily as well as the degenerin/ epithelial Na^+ channels (DEG/ENaC). Members of both families are widely expressed throughout the animal kingdom and are present in sensory neurones in invertebrates such as C*aenorhabditis elegans* (Ernstrom and Chalfie, 2002) or *Drosphila melanogaster* (Kernan et al., 1994; Kernan and Zuker, 1995) and vertebrates (reviewed by (Hu et al., 2006; Lewin and Moshourab, 2004)). (For further reviews see also (Goodman and Schwarz, 2003; Tobin and Bargmann, 2004).)

1.2 A molecular model for mechanotransduction

How are mechanosensitive ion channels gated? To date there are three main hypotheses for how mechanical forces activate mechanosensitive channels. The first and simplest model for mechanotransduction is composed only of the transducing ion channel itself that is directly gated by bilayer tension. No further accessory proteins are needed in such complexes. An example of this model is the prokaryotic stretch-activated channel MscL (Sukharev et al., 1994) in *Escherichia coli* that plays a role in turgor regulation. Controversial data discussing a similar mechanism for the mammalian ENaC channels, but without direct evidence *in vivo* are reviewed by (Hamill and Martinac, 2001). Other examples for stretch-activated channels in mammals include neuronal mechano-gated K+ channels such as TREK-1 (Patel et al., 1998) and TRAAK (Maingret et al., 1999). In a second model it is hypothesised that the release of extracellular ligands such as purinergic ATP could activate ion channels involved in mechanotransduction (reviewed by Burnstock: (Burnstock, 1999)). Members of the two classes of P2 purinoreceptors for ATP (ionotropic P2X receptors and G-protein-coupled P2Y receptors) are expressed by DRG neurones and are suggested to modulate sensory transmission in mammals (Chen et al., 1995; Nakamura and Strittmatter, 1996). However, such a mechanism would obviously not be fast enough to explain the very high speed of the response of a mechanoreceptor

to tactile vibratory stimulation for instance. Considering the speed of the signalling process in sensory neurone mechanotransduction (<1msec), (Corey and Hudspeth, 1979; O'Hagan et al., 2005; Walker et al., 2000) a third more complex model has been derived from studies on hair cells and invertebrate mechanoreceptors. Here the transducing ion channel is though to be tethered to the cytoskeleton and/or the extracellular matrix. Mechanical forces applied to the cell membrane are thought to be converted into tension between the tethers and therefore regulate the gating of the channel (reviewed by (Gillespie and Walker, 2001; Lewin and Moshourab, 2004)). Several candidate molecules that fit with this model have so far been identified in *C. elegans*, *D. melanogaster* and also in mammals (reviewed by (Goodman and Schwarz, 2003; Hu et al., 2006; Lewin and Moshourab, 2004; Tobin and Bargmann, 2004)).

1.2.1 The mechanotransduction model in C. elegans

A molecular model for cutaneous body touch in the nematode *C. elegans* has emerged from studies by Chalfie and Sulston twenty years ago who used classical mutagenesis approaches to screen mutated wild-type worms for gentle touch sensation along the body of the worms.

Chalfie and colleagues identified twelve *mec* (mechanosensory abnormal) genes responsible for touch cell function and another six important for touch cell development (Chalfie and Au, 1989; Chalfie and Sulston, 1981).

The central component in the mechanotransduction model derived from these studies is a mechanosensitive ion channel composed of MEC-4 and MEC-10, which are considered to form the pore of the heteromultimeric ion channel complex. The N- and the C-terminus of the channel proteins are cytoplasmic but their central regions are exposed to the extracellular side of the membrane and possibly attached to proteins of the ECM (Lai et al., 1996). Two accessory proteins (MEC-2 and MEC-6), which might be subunits of the ion channel itself, have been shown to modulate its activity (Chelur et al., 2002; Goodman et al., 2002). *mec-5* and *mec-9* encode ECM proteins, a collagen (MEC-5) and a EGF/Kunitz repeat protein (MEC-9) that may also interact with each other in the mantle. On the intracellular side the ion channel seems to be linked to a specialised 15- protofilament microtubule network formed by

MEC-12, an α-tubulin and MEC-7, a β-tubulin, which are also shown to be essential for mechanosensation in *C. elegans* (Huang et al., 1995), Fig. 1).

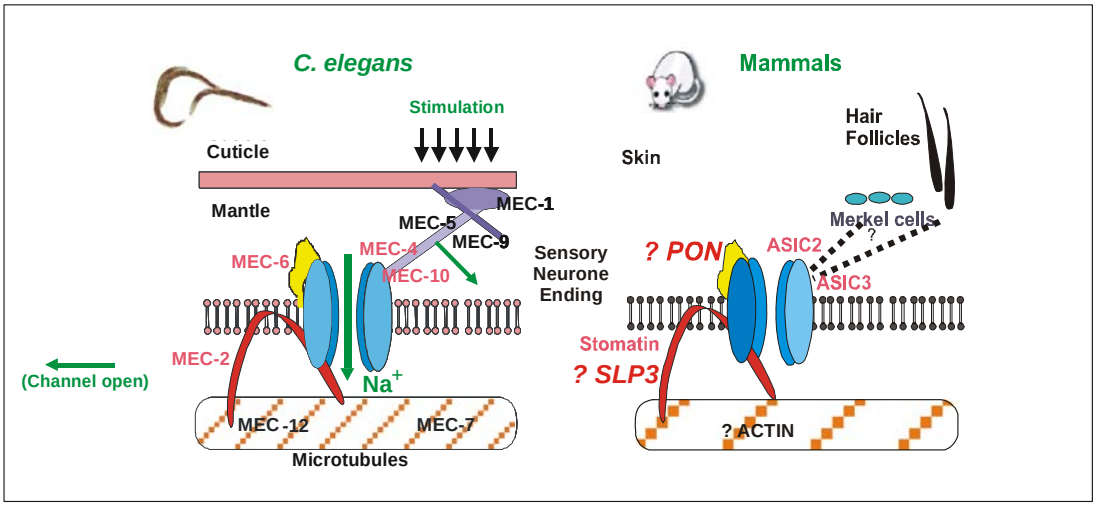

Figure 1 Molecular model of mechanotransduction in *C. elegans* and mammals
(adapted from Taveranakis & Driscoll, 1997). Possible arrangement of the *C.elegans* MEC proteins forming a mechanotransducing complex is shown. **Left:** The ion channel is formed by MEC-4, MEC-10 and MEC-6. Microtubules located close to the intracellular side of the membrane are formed by MEC-7 and MEC-12 and might be connected to the channel via MEC-2. Large extracellular domains of the channel are linked to the ECM proteins MEC-5 and MEC-9 that are suggested to pull on the channel proteins. **Right:** Several homologues of the *mec* genes have been identified in mice and humans. Their possible involvement in mechanotransduction is in the process of being elucidated.

In the absence of a mechanical stimulus the ion channel is normally closed. In response to mechanical forces the two rigid structures of the mechanotransduction complex, i.e. the extracellular mantle and the intracellular microtubule network, presumably move relative to one another and consequently the channel opens. For further reviews see also (Garcia-Anoveros et al., 1995; Tavernarakis et al., 1997) (Ernstrom and Chalfie, 2002).

Although this model remains mostly hypothetical, it is a useful tool to identify mammalian homologues. Several orthologue genes to those required for normal touch sensation in *C. elegans* have already been identified in mice and their possible involvement in mechanotransduction has in some cases been evaluated (Alvarez de la Rosa et al., 2002; Garcia-Anoveros et al., 2001; Mannsfeldt et al., 1999; Price et al., 2000; Price et al., 2001).

1.2.1.1 The mechanotransduction core complex in C. elegans

Of all *mec* genes identified in the mutagenesis screen by Chalfie and colleagues at least four genes, namely *mec-4, mec-10, mec-6* and *mec-2* are proposed to form a mechanotransduction core complex. The membrane protein products of these four genes are co-expressed in touch neurones in *C. elegans* (Chelur et al., 2002; Goodman et al., 2002) and can also co-immuniprecipitate with each other when expressed in CHO (Chinese hamster ovary) cells (Chelur et al., 2002).

When the "d"(denoted)-mutated forms of MEC-4 and MEC-10 are co-expressed in *Xenopus Laevis* oocytes both proteins form a heteromultimeric, constitutively open voltage-insensitive ion channel that generates amiloride sensitive Na^+ currents, which is a feature of DEG/ENaC family members. Interestingly, when either gene was expressed alone no such current could be generated (Goodman et al., 2002). Co-expression of MEC-4(d) and MEC-10(d) together with MEC-2, a stomatin-related protein, in a heterologous expression system enhances the current amplitude of the MEC-4(d)/MEC-10(d) ion channel ~ 40-fold and produces an amiloride sensitive current that is also seen in PLM cells expressing the wild-type MEC-4/MEC-10 ion channel. These data indicate that MEC-2 might have a regulatory function on MEC-4/MEC-10 ion channels (Goodman et al., 2002). *mec-6* encoding a single-pass membrane spanning protein with sequence similarities to vertebrate paraoxonase (PON) is also proposed to be a part of a mechanotransduction core complex in *C. elegans* blocking touch cell degeneration caused by *mec-4(d)* and *mec-10(d).* When MEC-6 is expressed together with MEC-4(d) and MEC-10(d) in *X. laevis* oocytes the current amplitude is enhanced ~40-fold. MEC-6 is co-localised with MEC-4 in the touch receptor cells of the worm and it is required for the punctuate expression pattern of MEC-4 in these cells. Co-expression of all four proteins in *X. laevis* oocytes increases the channel activity synergistically (Chelur et al., 2002).

In 2003 Suzuki et al. used Ca^{2+} imaging techniques to record responses to mechanical forces in *C. elegans* touch receptor neurones. Within 50 milliseconds following mechanical stimulation they found an increase in Ca^{2+} concentration that was absent in *mec-4* and *mec-10* mutants upon light touch application (Suzuki et al., 2003). Although this increase in Ca^{2+} is a secondary response to the activation of a

mechanically gated ion channel this experiment bolstered the hypothesis that MEC-4 and MEC-10 are required for transduction.

O'Hagen went on to provide more direct evidence through *in vivo* recordings from PLM cells required for behavioural responses to touch applied along the posterior part of *C. elegans*. Using a modified slit-worm preparation, in which the neurones remained intact during exposure to force application, he did whole cell recordings and found that forces less than 100nN elicited mechanoreceptor currents from the neurones with latencies less than one millisecond (O'Hagan et al., 2005). These currents could be blocked by the drug amiloride as expected for members of the DEC/ENaC ion channel family. When worms with a null mutation of *mec-4*, *mec-2* or *mec-6* were examined, no mechanosensitive currents could be recorded from the touch cells upon exposure to the maximum force of 11.5µN. This provides evidence for the hypothesis that these proteins are subunits of a sensory mechanotransduction complex (O'Hagan et al., 2005).

1.2.2 Candidate molecules involved in mechanotransduction in mammals

Recent attempts to elucidate the molecular basis of mechanotransduction in mammals have led to the identification of several candidate genes encoding mechanically gated ion channels or ion channel subunits that include members of both TRP and DEG/ENaC families (reviewed by (Goodman and Schwarz, 2003; Hu et al., 2006; Lewin and Moshourab, 2004; Tobin and Bargmann, 2004)). Considering the diversity of mechanosensitive receptor cells in mammals it is however also conceivable that there are multiple mechanisms underlying mechanotransduction.

1.2.2.1 Candidate ion channels

In mammals, the DEG/ENaC family consists of two major subfamilies, which include the acid sensing ion channels (ASIC) and ENaC. And it is possible that they might be involved in mechanotransduction. The ENaC channel is composed of three subunits (α, β and γ) and is expressed in apical membranes of transporting epithelia in lung, kidney and colon. The channel is constitutively open and its conductance is

controlled by several hormones (reviewed by (Kellenberger and Schild, 2002)). The channel is thought to play an essential role in controlling the Na^+ transport in epithelia in these organs (Kamynina and Staub, 2002).

The ENaC channel subunits β and γ (but not the pore forming α-subunit that in epithelia is responsible for the constitutively open state) are also found in arterial baroreceptor neurones. Antibodies against the γ-subunit of the channel stained the baroreceptor terminals in the aortic arch as well as in the carotid sinus, the putative site of mechanotransduction in baroreceptor sensory neurones detecting acute fluctuations in arterial pressure (Drummond et al., 2001). The β- and γ- subunits were also found to be expressed in medium and large size neurones of the lumbar DRGs, Merkel-cell-neurite complexes, Meißner's-like corpuscles and small lamellated corpuscles in rat hairless skin but since the pore forming α- subunit is not expressed it has been supposed that other channels play a role in mammalian touch sensation (Drummond et al., 2000).

Due to its homology to MEC-4 *C. elegans*, three members of the ASIC family, which are expressed in DRG neurones, are proposed to be good candidate molecules in sensory mechanotransduction in mammals. ASIC2 is expressed in many large diameter neurones of the DRG that include low threshold mechanoreceptos (LTM). Antibodies against ASIC2 label endings within Meissner's corpuscles, Merkel cells, hair follicles and some myelinated free nerve endings in the skin (Garcia-Anoveros et al., 2001). Deletion of the *asic2* gene reduces the sensitivity of low threshold mechanoreceptors in the hairy skin in mice, although it did not eliminate the response of rapidly-adapting mechanoreceptors (RAM) completely (Price et al., 2000).

ASIC3 is co-expressed with ASIC2 in large diameter sensory neurones, but it also appears in medium and small size neurones in the DRG. In addition to ASIC2 the ASIC3 protein is also located in nociceptive intra-epidermal sensory endings in the skin (Price et al., 2001). *asic3* gene deletion in mice results in an increased sensitivity of RAM fibres but on the other hand mechanical nociceptors lose sensitivity. Furthermore, the response of acid- and heat-sensitive nociceptors is reduced in ASIC3 mutant mice indicating that ASIC3 may be a component of a heteromulimeric transduction channel in mice (Price et al., 2001) (Moshourab, R. Thesis 2006). The third member of the ASIC family found in mammalian sensory neurones is ASIC1

that is expressed in most somatosensory neurones in rat (Alvarez de la Rosa et al., 2002). The mutation of the *asic1* gene in mice results in an increased firing rate in colonic and gastroesophageal mechanoreceptors. However, cutaneous mechanoreceptors remain unaffected indicating that different mechanisms for mechanotransduction may exist in the gut and skin (Page et al., 2004).

1.2.2.2 Candidate regulatory proteins

Recently, studies by O'Hagen and colleagues have provided evidence that in a sensory mechanotransduction core complex MEC-2 and MEC-6 are subunits of the MEC-4/MEC-10 ion channel expressed in touch receptor cells in *C. elegans* (O'Hagan et al., 2005). Using a slit-worm preparation they were able to record mechanoreceptor currents (MRC) from wild-type touch neurones *in vivo*. Loss of *mec-2* or *mec-6* in mutant worms resulted in the elimination of MRCs supporting the hypothesis that both proteins function as subunits of a mechanically gated ion channel in these animals. For both of the genes, orthologues are also known in mammals and will be described here.

1.2.2.2.1 Mammalian homologues of MEC-2

The closest mammalian homologue to MEC-2 is stomatin also known as band 7.2b protein. Stomatin is one of the major integral membrane proteins of human erythrocytes which is absent in patients with "overhydrated hereditary stomatocytosis" (Hiebl-Dirschmied et al., 1991b; Stewart et al., 1992; Wang et al., 1991), a congenital haemolytic anaemia characterised by its mouth-shaped erythrocytes that exhibit a forty-fold increase in membrane permeability for Na^+ and K^+ ions. Previously, studies on stomatin showed that it is widely expressed in various human tissues (Gallagher and Forget, 1995) (Stewart et al., 1992) and amongst others present in ciliated cells of the human airway epithelium (Fricke et al., 2000; Fricke et al., 2003).

The stomatin protein contains a highly charged, short N-terminus, followed by a hydrophobic stretch, representing the putative membrane insertion domain and a

large hydrophilic C-terminal domain. The N- as well as the C-terminus is cytoplasmic in orientation indicating a monotopic structure of the protein inserted in the phospholipid membrane in a hairpin-like manner (Hiebl-Dirschmied et al., 1991b; Salzer et al., 1993). There is also evidence that stomatin forms homooligomers, presumably with its C-terminal domain (Snyers et al., 1998). One of the three cysteine residues of stomatin is predicted to be a palmitoylation site that in conjunction with its putative oligomeric nature might enhance its membrane affinity (Snyers et al., 1999). Stomatin null mutant mice have normal erythrocyte morphology, monovalent cation content suggesting that stomatin deficiency alone is not sufficient to cause overhydrated hereditary stomatocytosis (Zhu et al., 1999). Moreover, genetic linkage analyses could exclude *stomatin* as candidate gene for this disease (Innes et al., 1999).

In analogy to the regulatory role of MEC-2 in cutaneous mechanotransduction in *C. elegans* (Goodman et al., 2002; Huang et al., 1995) and with regard to its expression in sensory neurones of the DRG in mice (Mannsfeldt et al., 1999) stomatin has been proposed to play a functional role also in vertebrate sensory mechanotranduction. Interestingly, stomatin expression is upregulated during development when sensory neurones innervate their peripheral targets (Mannsfeldt et al., 1999). However, although stomatin is expressed by all sensory neurones of the DRG, *stomatin* deletion in mice revealed only a minor mechanosensory phenotype (Martinez-Salgado, Benckendorff, Wetzel & Lewin MS in prep).

Stomatin and MEC-2 are both members of the stomatin domain family containing a central region that is strikingly similar to the prohibitin family of mitochondrial proteins (Dell'Orco et al., 1996), the caveolae-associated flotillins (Bickel et al., 1997) and the bacterial plasma membrane proteins HflK and HflC (Noble et al., 1993). This region was thus named the SPFH (stomatin/ prohibitin/flotillin/ HflK/C) domain and is thought to regulate interactions with membrane-associated proteins and might also be required for oligomerisation of the proteins.

The dendrogram in figure 2 illustrates the evolutionary relatedness of the family members.

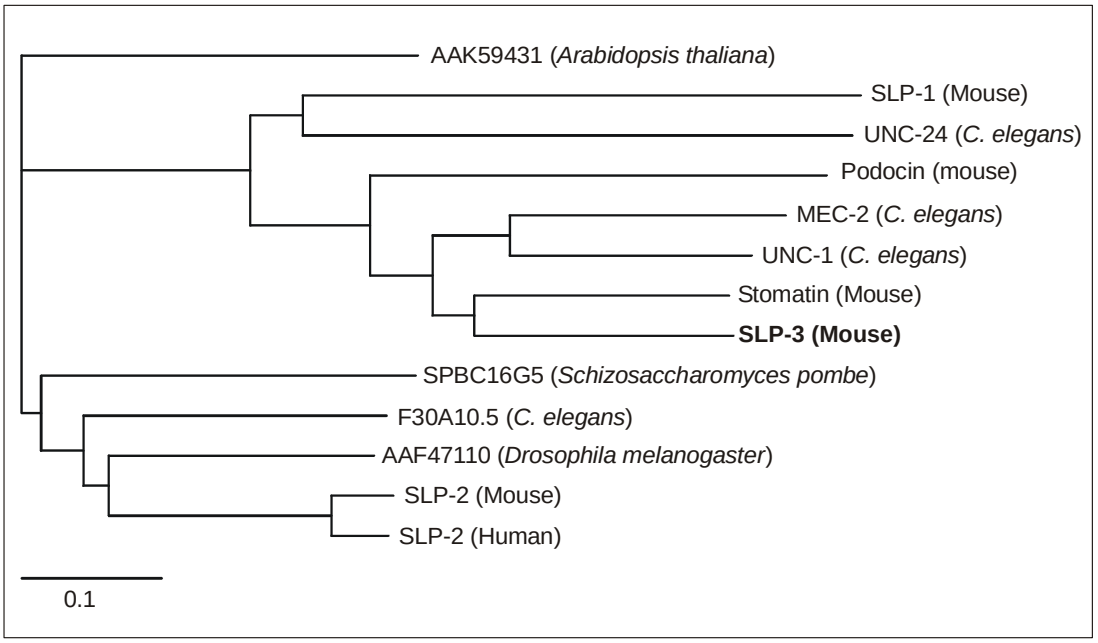

Figure 2 Phylogenetic tree of stomatin-like proteins.
The evolutionary relationships amongst the family members of the stomatin-domain family are illustrated. All members share a highly conserved central region, named after the initials of their related protein families SPFH (stomatin, prohibitin, flotillin, and HflK/C).

Apart from *mec-2* other *stomatin*-like genes have been genetically characterized in *C. elegans*, namely *unc-24* and *unc-1*. Both encode proteins that are expressed in the nervous system and required for normal locomotion in the worm but UNC-1 furthermore controls anaesthetic sensitivity (Rajaram et al., 1998). Unc-24 co-localises with MEC-2 *in vivo* and can be co-immunoprecipitated with MEC-2 and MEC-4 in *X. leavis* oocytes. It is proposed to enhance touch insensitivity of temperature-sensitive alleles of *mec-4* and *mec-6* (Zhang et al., 2004).

Other proteins belonging to the stomatin family have been identified, which are mainly expressed in brain. The human stomatin-like protein 1 (hSLP1), which is expressed at high levels in the basal ganglia, encodes a bipartite protein with a stomatin-like domain at the N-terminus and a large C-terminal part similar to non-specific lipid transfer proteins (nsLTPs). Human SLP2 (stomatin-like protein 2) shares a 20 % overall similarity to human stomatin and its tissue specific expression overlaps widely with that of SLP1, but unlike SLP1 it is also expressed in human erythrocytes. The unusual feature of SLP2 is the lack of the $NH_{(2)}$-terminal hydrophobic domain found in other stomatin homologues (Wang and Morrow, 2000). Using RT-PCR and Northern blot techniques, mRNA transcripts of *slp2* could also be found in mice where it is expressed in brain, detectable in cortex, cerebellum

and hippocampus but also in all sensory neurones of the DRG (Paul Heppenstall, personal communication).
Another very close homologue of MEC-2 in mammals is SLP3 (Goldstein et al., 2003) (Kobayakawa et al., 2002), which is also known to be expressed by olfactory neurones. Both proteins share the hairpin-like structure in the membrane. At the amino acid sequence level stomatin and SLP3 are the most related proteins to MEC-2, showing 65% identity and 85% similarity to MEC-2 in its core region (Mannsfeldt, A. Thesis 1999) (Huang et al., 1995). As found by *in situ* hybridisation *slp3* mRNA is expressed in neurones of the peripheral and central nervous system (Mannsfeldt et al., 1999) (Wetzel et al., Nature 2006 in press). Thus, this protein may be a good candidate for being a regulatory subunit in a mechanotransduction complex in mammals.

1.2.2.2.2 Mammalian homologues of MEC-6

MEC-6 in *C. elegans* encodes a 377 amino acid polypeptide showing 25% identity and 45% similarity over a stretch of 225 amino acids at its C-terminus to mammalian paraoxonase (PON). MEC-6 has been shown to possess a single-pass membrane-spanning domain with a short cytoplasmic N-terminus and a large extracellular C-terminal domain. In *C. elegans* it is expressed in neurones including all six touch receptor cells and in muscle (Chelur et al., 2002).
Three *paraoxonase* genes (*pon1*, *pon2* and *pon3*) have been described in mammals. All three *pon* genes are located adjacent to each other on chromosome 7 in humans and on chromosome 6 in mice, thus they seem to be a result of gene duplication. At the amino acid level PON1, PON2 and PON3 show a high similarity between mammalian species (Primo-Parmo et al., 1996). Whereas PON1 and PON3 are primarily expressed in liver (Reddy et al., 2001) PON2 is widely expressed in a number of tissues, including liver, kidney and testes but also in the brain (Mochizuki et al., 1998).
Human serum PON1 and also PON3 are high-density lipoprotein (HDL)-bound ester hydrolases that catalyse the hydrolysis of a number of organic esters and protect low-density lipoprotein (LDL) from oxidation (Harel et al., 2004) (Reddy et al., 2001). PON2 on the other hand is not HDL associated but may function similar like PON1

and PON3 (Reddy et al., 2001) (Ng et al., 2001) in preventing cell-mediated oxidative modification of LDL. However, little is known about the role of the *pon* gene products in human physiology and pathology. Thus, considering the role of MEC-6 in *C. elegans* a possible role for paraoxonase in sensory neurone mechanotransduction will be investigated in more detail in this thesis.

1.3 Cutaneous mechanoreceptors in mice

Cutaneous sense organs represent a wide range of various receptors responsible for detecting mechanical, thermal or noxious stimuli applied to the body surface. Skin organisation is highly complex and there are three types of skin: glabrous, hairy and mucocutaneous, which all have a distinctive internal structure and contain their own assembly of mechanoreceptors. Cutaneous mechanoreceptor classification is based on the morphology and physiology of receptors and in addition their functional properties can be linked to neurochemical and biophysical features as well, for reviews see (Koerber, 1992; Lawson, 1992). Three types of morphological distinctive receptors are common to glabrous and hairy skin: Pacinian corpuscles, Merkel disc and free nerve endings. Meissner's corpuscles on the other hand are exclusively found in the primate glabrous skin.

Physiologically, two distinct kinds of responses are in principle possible, either afferent fibres discharge only during stimulus application, i.e. the movement of a mechanical stimulus is produced as a dynamic response, or they continue firing during the static maintenance of a mechanical stimulus as well. Receptors that are preferentially excited during the movement are called rapidly-adapting mechanoreceptors (RAM), whereas those receptors responding to both phases of the stimulation are defined as slowly-adapting mechanoreceptors (SAM).

In mammals the cell bodies of sensory neurones that are able to respond to mechanical stimuli are located in the DRG and several cranial ganglia.

Large diameter neurones in the DRG are thickly myelinated Aβ-fibres that conduct very rapidly (>10 m/s in mice, (Koltzenburg et al., 1997)) and represent low threshold mechanoreceptors that can be divided into two groups, namely RAM and SAM (Fig. 3). A-mechanonociceptors (AM) and D-hairs (Fig. 3) have medium size

axons that are thinly myelinated conducting in the Aδ-range with velocities between 1-10 m/s in mice (Koltzenburg et al., 1997). AM fibres are excitable by either pressure of very high intensity, pinching the epidermis or cutting the skin with sharp objects, whereas D-hairs belong to low threshold mechanoreceptors and conduct exclusively during the movement phase of the mechanical stimulus.

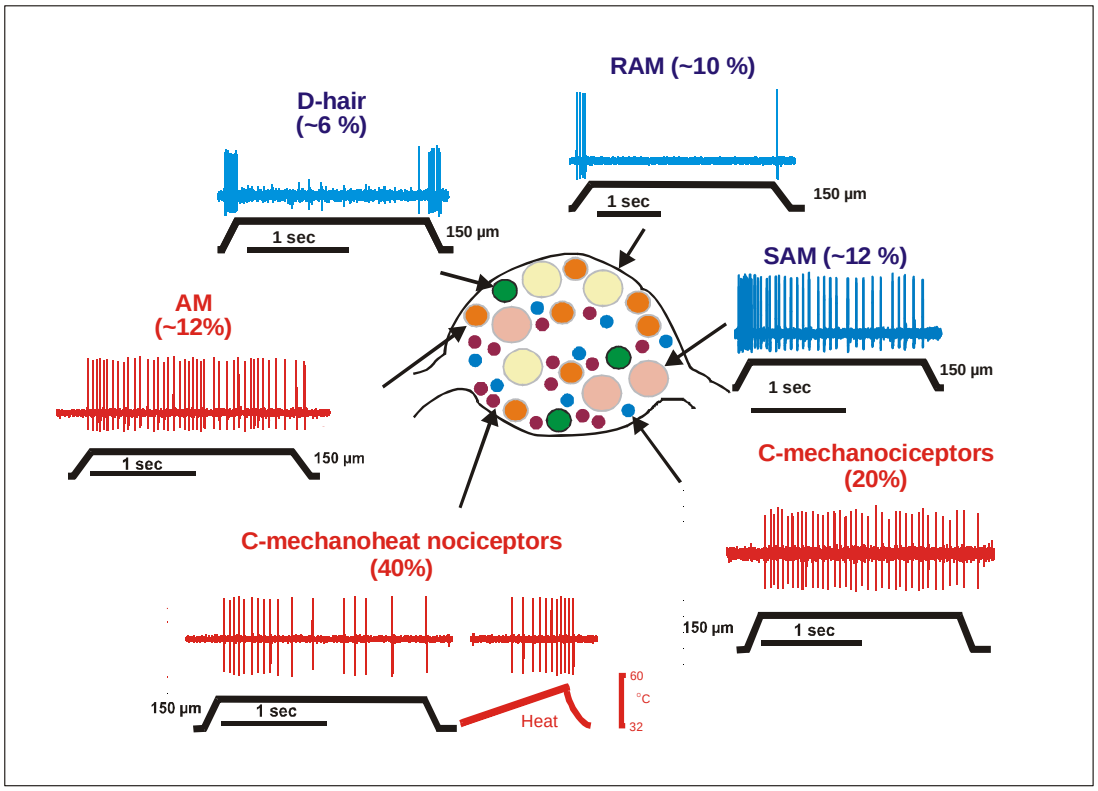

Figure 3 Mechanoreceptors found in mouse skin innervated by the saphenous nerve. Typical response properties of mouse mechanoreceptors from the saphenous nerve to a standardised ramp and hold indentation stimulus at 150µm. A schematic drawing of a dorsal root ganglion containing myelinated and non-myelinated neurones with distinct cell diameters is shown in the centre. Low threshold mechanoreceptors that all respond robustly to the ramp phase are depicted in blue and high threshold mechanonociceptors responding primarily to static indentation of the skin are shown in red. The approximate incidence of the mechanoreceptor class is indicated next to its name.

C-fibres (Fig.3) that make up the largest group of mechanoreceptors in mice (60%) lack a myelin sheath and therefore conduct very slowly, i.e. below 1.0 m/s in mice (Koltzenburg et al., 1997). They can roughly be subdivided into two groups: Firstly, C-mechanoheat (C-MH) fibres respond to both noxious mechanical and thermal stimuli and are thus so called polymodal receptors. They can also be activated, or at least sensitised, by a wide range of algesic chemicals such as capsaicin. Secondly, there is a substantial number of C-fibres responding to mechanical, but not to thermal

stimulation. These fibres are classified as C-mechanonociceptors (C-M) (Fleischer et al., 1983; Kress et al., 1992; Lewin and Mendell, 1994).

1.4 Aims

Recent studies have provided evidence for the existence of a mechanotransduction core complex in *C. elegans* formed by MEC-4, MEC-10, MEC-6 and MEC-2. On the basis of homology studies several orthologues of the *mec* genes products have also been identified in mammals including ion channels such as ASIC2 or ASIC3 (homologues of MEC-4) or accessory proteins like stomatin and SLP3 (homologue to MEC-2) or paraoxonase (homologue to MEC-6) for example.

The main work was concentrated on the protein SLP3 (stomatin-like protein 3) and its role in sensory mechanotransduction in mice. A mouse model was generated, in which the *slp3* gene was mutated. SLP3 mutant mice were characterised in detail and extensive studies of the function of individual mechanoreceptor subclasses using the *in vitro* skin nerve preparation for extracellular recordings from isolated sensory afferents in the skin were performed. Analyses of $SLP3^{-/-}$ mice also included immunohistochemical studies and extensive behavioural experiments that in the first instance had to be developed for wild-type mice. Since stomatin and SLP3 are very similar at the amino acid level and interact when heterologous expressed in HEK293 cells a double mutant mouse line lacking both proteins was generated and analysed using electrophysiological and immunohistochemical techniques. A smaller project addressed the question of whether the MEC-6 homologue paraoxonase could also be a good candidate molecule involved in sensory neurone mechanotransduction in mammals. Therefore biophysical analyses and classical biochemical approaches like Western blot analyses for example were carried out.