Developmental Biology 327 (2009) 121–131

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

The role of Notch signalling and numb function in mechanosensory organ formation in the spider Cupiennius salei

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article info

Article history:
Received for publication 1 October 2008
Revised 2 December 2008
Accepted 3 December 2008
Available online 16 December 2008

Keywords:
Peripheral neurogenesis
Mechanosensory organs
Notch signalling
Numb
Prospero
Delta
Cell fate
Chelicerate
Cupiennius salei

abstract

In the spider Cupiennius salei the mechanosensory organs of the legs are generated from epithelial sensory precursor groups which are specified by elevated levels of the achaete–scute homologues CsASH1 and CsASH2. Neural precursors delaminate from the groups and occupy positions basal and proximal to the accessory cells which remain in the epithelium. Here we analyse the role of Notch signalling and numb function in the development of the mechanosensory organs of the spider. We show that Notch signalling is required for several processes: the selection of the sensory precursor groups, the maintenance of undifferentiated sensory precursors, the binary cell fate decision between accessory and neural fate and the differentiation of sensory neurons. Our data suggest that Numb antagonises Notch signalling in the neural precursors, which results in activation of the neural cell fate determinant Prospero and delamination of the neural precursors from the epithelium. Prospero is expressed de novo in sensory neural precursors and we assume that the expression of the gene is regulated by the Notch to Numb ratio within the sensory precursors. Interestingly, the spider numb RNAi phenotype resembles the numb/numblike loss of function phenotypes in the mammalian nervous system, indicating that the interaction between Notch signalling and Numb might play a similar role in both systems.

Introduction

Arthropod sensory systems consist of a large number and great diversity of small sensory organs (reviewed by Hartenstein, 2005). Each sensillum is innervated by one or several neurons which respond to specific stimuli. The stimuli are received by modified epidermal cells and transmitted to the sensory neurons, which results in an action potential that is transmitted via the axons towards the central nervous system (CNS).

Despite structural differences, the cellular composition and arrangement of the large majority of arthropod sense organs is very similar (Hallberg and Hansson, 1999; Hartenstein, 2005). They contain bipolar sensory neurons that are surrounded by inner and outer accessory cells (Fig. 1A). The inner accessory cells, the sheath cells, enwrap the dendrite(s), while the outer accessory cells secrete cuticle in the shape of hairs and sockets (external sense organs) or form ligaments that attach the sensory neurons to the body wall (subepidermal stretch receptors). The common ground plan suggests that arthropod sensilla derive from the diversification of an ancestral sense organ (Lai and Orgogozo, 2004).

The molecular processes underlying arthropod sensory organ (SO) development have only been studied in insects, mainly Drosophila melanogaster (reviewed by Hartenstein, 2005; Lai and Orgogozo, 2004). Individual sense organ progenitor cells (SOPs) are selected from the ectoderm and undergo stereotyped asymmetric cell divisions before differentiation to generate the neuron(s), glia and accessory cells of the sensillum (Fig. 1B). The first step in SO development is the expression of the proneural genes. These encode basic-helix–loop–helix (bHLH) transcription factors, which confer SOP potential to cells and/or specify sensory organ subtype identity. Initially the proneural genes are expressed in large groups of epithelial cells, the proneural clusters, but expression is reduced to single cells through lateral inhibition mediated by the Notch signalling pathway. The different fates within a sensory organ lineage are determined by combinations of intrinsic and extrinsic factors (reviewed by Hartenstein, 2005). The binary cell fate choice between the two SOP daughter cells (pIIa and pIIb) depends on the activity of Numb and members of the Notch signalling pathway (Guo et al., 1996). Numb prevents Notch signalling in the pIIb cell, which is essential for the entrance of pIIb into the neural pathway. Binary cell fate decisions mediated by Notch and Numb occur again among the progeny of pIIa and pIIb — the neural precursor pIIIb and the glia, the neuron and sheath cell and the hair and socket cells, respectively (Guo et al., 1996).

Recently, we presented the first study on the development of larval SOs on the legs of the spider Cupiennius salei (Stollewerk and Seyfarth, 2008). In contrast to insects, groups of sensory organ precursors (SPGs) are specified in the spider leg epithelium. We identified 3

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doi:10.1016/j.ydbio.2008.12.004

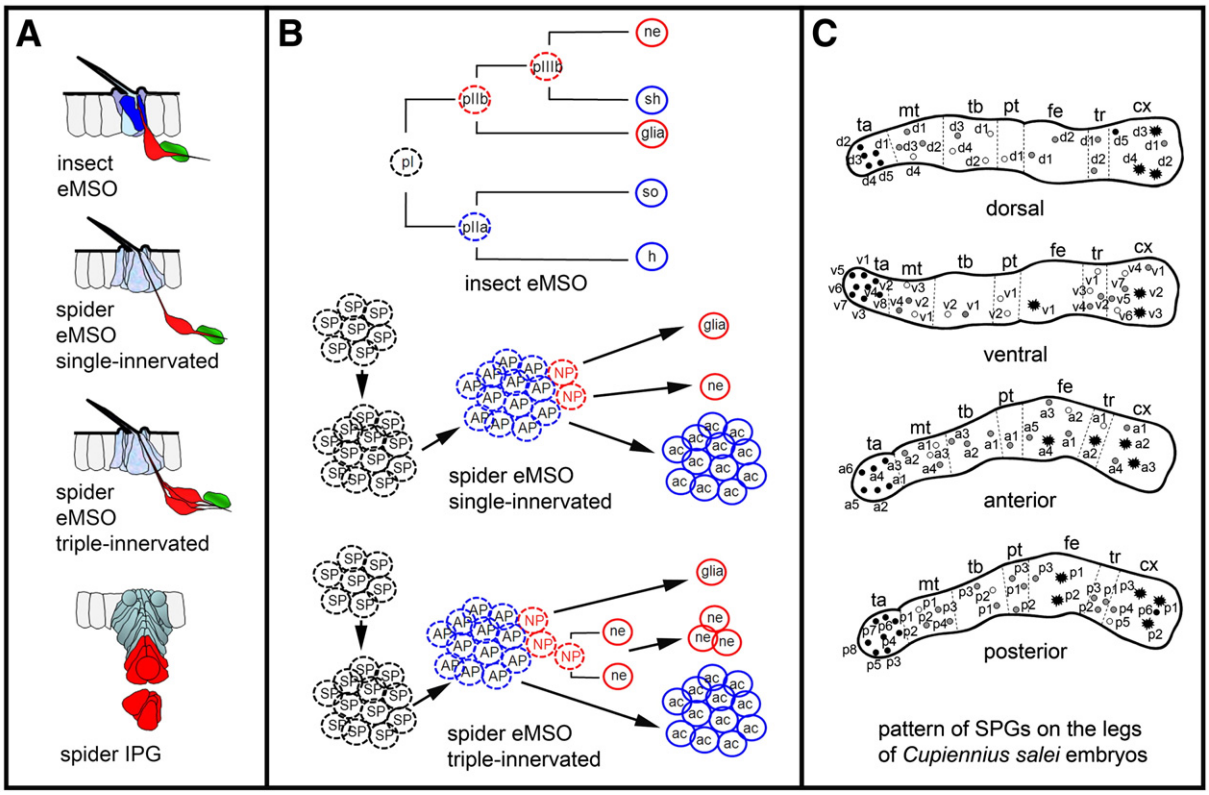

Fig. 1. Cell composition, formation and pattern of MSOs in the spider Cupiennius salei. (A) Comparison of the cellular composition of eMSOs in insects and in the spider. The schematic drawing of the insect sense organ represents the proposed common ancestral lineage of eMSOs in insects described in (Lai and Orgogozo, 2004). In insects eMSOs consist of 5 cells: a hair (light-blue), a socket (purple) and a sheath (blue) cell (accessory cells) which remain in the epithelium and a neuron (red) and a glial cell (green) which delaminate. The glial cell might undergo apoptosis as has been shown in the thoracic microchaete lineage of Drosophila melanogaster (Fichelson and Gho, 2003). In the spider the developing single- and triple-innervated eMSOs consist of 12 accessory cells (light-blue-purple), 1 presumptive glial cell (green) and 1 or 3 neurons, respectively (red). IPGs do not differentiate during embryogenesis but they generate neural precursors (red). (B) In insects eMSOs derive from single SOPs which divide asymmetrically, whereas in the spider groups of sensory precursors generate the MSOs. ac, accessory cell, ap, accessory precursor, glia, glial cell, h, hair cell, ne, neuron, pI, SOP, pIIa, hair/socket precursor, pIIb and pIIIb, neural precursors, sh, shaft cell, so, socket cell, sp, sensory precursor. (C) Distribution of the characterised SPGs on the dorsal, ventral, anterior, posterior side of the leg. Proximal is to the right and anterior is at the top for the dorsal and ventral side of the leg, dorsal is at the top for the anterior and posterior side of the leg. The dashed lines indicate the segmental borders. Large black dots represent IPGs. The grey dots represent the developing triple-innervated eMSOs, the light grey dots the single-innervated eMSOs. Black dots represent SPGs that were not classified. cx, coxa; tr, trochanter; fe, femur; pt, patella; tb, tibia; mt, metatarsus; ta, tarsus.

different types of SPGs, which are located at stereotyped positions (Figs. 1B, C). Two types give rise to single- and triple-innervated external mechanosensory organs (eMSOs), whereas the third type presumably generates internal mechanosensory joint receptors, which do not differentiate until the 2nd larval stage (Fig. 1A). Functional studies revealed that both spider achaete–scute homologues (CsASH1 and CsASH2) are responsible for the formation of all eMSOs of the legs and participate in the development of the presumptive internal joint receptors. Both the single- and triple-innervated external mechanosensory precursor groups (eMSPGs) consist of 12 accessory cells, which remain in the epithelium and give rise to the hair, socket and sheath cells (Fig. 1B). The single innervated eMSPGs produce two neural cells, a neuron and a presumptive glia, while the triple-innervated eMSPGs generate three neurons and a presumptive glial cell. The neural cells delaminate and occupy a position basal and proximal to the accessory cells of the SOs (Fig. 1A).

The presumptive internal joint receptor precursor groups (IPGs) initially consist of 15 to 30 cells and the group sizes increase to 60 to 80 cells until the end of embryogenesis. Although the IPGs do not differentiate into functional SOs until the 2nd larval stage, they produce large numbers of neural precursor cells during embryogenesis (Fig. 1A). The neural precursors delaminate individually from the epithelial IPGs and form tight clusters in the centre of the leg (basal), together with neural precursor cells from neighbouring IPGs.

The formation of groups of SO precursors raises the question of how individual precursor cells are selected for the various cell fates. Since the members of the Notch signalling pathway and numb play a vital role in SOP selection and sensory cell type identity in Drosophila, we analysed the function of these genes in MSO development in the spider C. salei.

Materials and methods

C. salei stock

Fertilised female spiders of the Central American wandering spider C. salei Keyserling (Chelicerata, Arachnida, Araneae, Ctenidae) were obtained from Ernst-August Seyfarth's laboratory colony at the Institute for Cell Biology and Neuroscience, J. W. Goethe-Universität, Frankfurt am Main, Germany. Embryos were collected as described before (Stollewerk et al., 2001).

Numb cloning

The following primers for numb were used: numbF1, CNW SNM RNC AYC ART GGC A; numbR1, GTN ACN CCR CAY TCY TTX TC; numbF2, GTN GAY CAR ACN ATH GAR AAR; numbR2, AAN GCR CAN CLN ACX GCR TG. NumbF2 and numbR2 were used for the nested PCR. Clones were obtained using the pZero vector system (Invitrogen). The resulting DNA fragments were sequenced to confirm their identity. A

Table 1
RNAi phenotypes

RNAi	WT	Affected SPGs	Missing SPGs	Phenotypes as listed	Reduced neurons	Increased neural cells	SPG morphology affected + reduced accessory cells	Cell clots	Delamination affected	Premature differentiation	Increased in no. of SPGs	Neural differentiation affected
Dl 1	37	63	35	65	3	31[a]	82	–	–	54[b]	24	
Dl 2	5	95	42	58	8	13[a]	95	–	–	48[b]	3	
N	1	99	71	29	49	3[a]	91	–	–			15[c]
nb	2	98	63	37	55	2[a]	–	50	65[b]			
GFP	100	–	–	–	–	–	–	–	–	–	–	

The table shows the percentages of SPGs in the different categories of observed phenotypes after Delta 1, Delta 2, Notch and numb ds RNA injection. Total number of SPGs analysed: Delta 1 RNAi, 270; Delta 2 RNAi, 490, Notch RNAi, 617; numb RNAi, 426; control (ds GFP RNA), 300 (not shown). All SPGs were present in control injected embryos and did not show any of the phenotypes listed in the table. Note that individual SPGs frequently fell into two or more categories so that the overall percentage does not add up to 100% in the columns to the right of 'phenotypes as listed'. WT: SPGs exhibit the same phenotype as wildtype and control injected embryos. Affected SPGs: SPGs show listed phenotypes or are missing. Missing SPGs: neural and accessory cells are absent in SPGs. Phenotypes as listed: SPGs that are present after RNAi treatment and show one or several of the phenotypes listed in the columns to the right. Reduced neurons: SPGs that express Prospero in less than 3 neurons (developing triple-innervated eMSOs) or 0 neurons (developing single-innervated eMSOs) or less than 8 neural precursors (IPGs). The number of neural precursors produced by individual IPGs is not known, however 10 to 15 neural precursors are continuously located close to each IPG in untreated and control injected embryos (see Fig. 6D). We only classified IPGs with less than 8 Prospero positive cells with the phenotype 'reduced neural cells'. Increased neural cells: to exclude incorrect counting owing to developmental delays we only took into account eMSPGs with increased numbers of neural cells that exceeded the maximal number of Prospero expressing cells during development (see Fig. 1B), i.e. eMSPGs that express Prospero in more than 4 neural cells (developing triple-innervated eMSOs) or more than 2 neural cells (developing single-innervated eMSOs). SPG morphology affected and reduced accessory precursor cells: in this category SPGs do not show the typical epithelial morphology (e.g. Fig. 4C) and accessory precursor cells are severely reduced (e.g. Fig. 4E). Cell clots: sensory precursors form cell clots. Delamination affected: Neural precursors remain attached to IPGs. Premature differentiation: Prospero positive cells of IPGs develop axons and dendrites. Increase in number of SPGs: 24% (Dl1) and 3% (Dl2) increase in SPGs (control = 100%). Neural differentiation affected: Prospero positive cells do not develop axons and dendrites and do not adhere to each other.

[a] eMSPGs only.
[b] IPGs only.
[c] Developing triple-innervated eMSPGs and IPGs only.

larger fragment of C. salei numb was obtained by 5′ rapid amplification of cDNA ends (5′/3′ RACE kit, 2nd generation, Roche). GenBank accession number: FJ025987.

Immunohistochemistry, in situ hybridisation and stainings

We used the following primary antibodies: rat anti-Cupiennius salei Prospero (1:200; Weller and Tautz, 2003), rabbit anti-human activated caspase-3 (1:50, Cell Signalling Technology), rabbit anti-phospho-Histon H3 (1:300; Upstate), sheep anti-digoxigenin fab fragments, AP conjugated (1:2000, Roche). Fluorescent secondary antibodies (1:250) were purchased from Jackson Immunoresearch Laboratories. Phalloidin-FITC (1:20) was purchased from Molecular Probes. In situ hybridisations, phalloidin-FITC stainings and antibody stainings were performed on whole-mounts as described before (Stollewerk et al., 2001). Fluorescent images were analysed under the Leica TCS SPII confocal microscope and processed using Leica Confocal software and Adobe Photoshop.

RNA interference

Double-stranded (ds) RNA was prepared from CsNotch, CsDelta1, 2 and Csnumb cDNA clones, respectively, by in vitro transcription using both Sp6 and T7 RNA Polymerases to generate sense and anti-sense RNA. The production of ds RNA was verified by gel electrophoresis. Since it has been shown that siRNA sequences from within 100 nucleotides of the 5′ termini of coding sequences had low chances for off-target reactivity (Qiu et al., 2005), we used the 5′ regions of all four genes as templates for the generation of ds RNA. 2 μl of ds CsNotch, CsDelta1, 2 and Csnumb RNA, respectively, were used for injection of approximately 300 embryos per RNA interference experiment. Ds GFP RNA was used for control injections. Embryos were injected before formation of the germ band (about 72 h) and fixed and analysed after 350 h of development, when all characterised SPGs were present in untreated embryos. We stained ds RNA injected embryos with DIG-labelled CsNotch, CsDelta1, 2 and Csnumb probes, respectively, and verified that the mRNA transcripts were significantly reduced or absent in the corresponding experimental embryos while they were unchanged in ds GFP RNA injected embryos. All SPGs were present in control injected embryos and did not show any of the phenotypes listed in Table 1. The SPGs of the experimental embryos fell consistently into the classes listed in Table 1.

Statistical analysis

Data from the experiments on eMSPGs and IPGs were analysed separately. Our data violated the assumptions of parametric methods from the generalised linear model family, so we adopted more robust non-parametric tests. The Kruskal–Wallis test was used to test for an overall effect of RNAi used on the number of apoptotic cells across all categories. The Wilcoxon two-sample test was then used to compare apoptotic cell counts for each different RNAi treatment with the control counts. Corrections for multiple comparisons used a sequentially-rejected Bonnferoni method. See Sokal and Rohlf (1995, pp 240–242, 423–434) for details of tests; all statistical analyses were performed using the R software (Team, 2007).

Results

Expression patterns of members of the Notch signalling pathway

The dual function of the neurogenic genes Notch and Delta in the selection of SOPs and the specification of the various cell fates within the Drosophila SO lineage is reflected in the expression patterns of the genes. Before selection of the SOPs the neurogenic genes are expressed at low levels in all epithelial cells in regions of SO formation (e.g. wing disc, leg disc). Higher levels of Delta expression are restricted to the proneural clusters, while strong Notch expression is largely complementary to Delta. After down-regulation of the proneural genes to single cells of the proneural clusters, both Delta and Notch are strongly expressed in the SOPs and subsequently in all progeny.

In the spider, CsNotch and CsDelta 1 and 2 are ubiquitously expressed in the leg epithelium prior to the formation of SPGs (mid-embryogenesis, 160 h of development; data not shown). We analysed the expression patterns of the neurogenic genes between 220 and 350 h of development in order to correlate their expression with the formation of the characterised SPGs (Stollewerk and Seyfarth, 2008) (Fig. 1C). At 220 h the neurogenic genes are expressed at low levels in all epithelial cells of the leg (Figs. 2A, C, E). In addition, transcripts

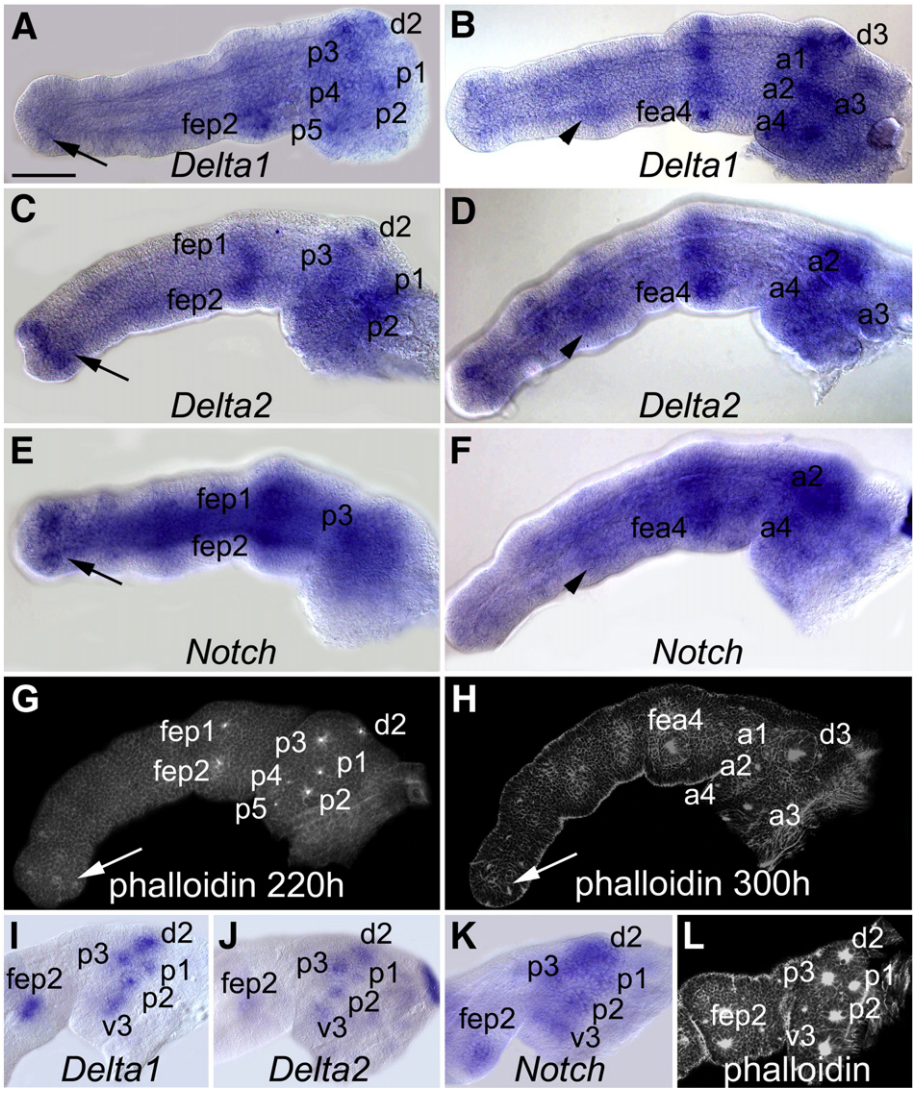

Fig. 2. Expression pattern of the neurogenic genes in the legs. Light microscopic images of in situ hybridisations of embryos stained with DIG-labelled probes (A–F, I–K) and phalloidin-FITC (G, H, L). A, C, E, G, I–L, posterior side of the legs, B,D,F,H, anterior side of legs. (A, C, E) At 220 h the neurogenic genes are expressed at low levels in the whole leg epithelium; higher levels of transcripts accumulate in the SPGs (arrows). (B, D, F) At 300 h the neurogenic genes show the same expression pattern in the IPGs (a1–a4) but accumulate in areas of eMSPG formation where they have not been up-regulated before (arrowheads). (G, H) Position of SPGs (bright spots) at 220 h and 300 h, respectively; arrowheads point to tarsal SPGs. (I–K) At about 350 h the neurogenic genes are still strongly expressed in the IPGs, whereas transcripts are down-regulated in the remaining cells of the leg. (L) Position of SPGs at 350 h. a1–4, coxal SPGs cxa1–4; d2–3, cxd2–3; p1–5, cxp1–5; v3, cxv3 (see Fig. 1C for nomenclature). Scale bar: (A) 80 μm.

accumulate in groups of cells that correspond to the positions of SPGs (Figs. 2A, C, E; compare to Figs. 2G and 1C). All genes show highly dynamic expression patterns in the regions where eMSPGs form, while they are strongly expressed in the IPGs from about their time of formation (220 h) until the end of embryogenesis (=360 h; Figs. 2I–K). Transcripts of all genes accumulate in all SPGs, although at different times, e.g. in Fig. 2 CsDelta1 is up-regulated in the eMSPGs cxp4 and cxp5 at 220 h, while CsDelta2 and CsNotch are uniformly expressed in this area.

At 250 h, 83% (76 of 91 per leg) of the characterised eMSPGs are present in the spider leg (see Fig. 1C). Individual cells in the groups express the neural cell fate determinant Prospero, indicating that sensory precursor cells have already entered the neural pathway. Between 250 h and 320 h CsNotch and CsDelta 1 and 2 continue to be expressed at low levels in all leg epithelial cells and stronger expression is visible in areas where the genes have not been up-regulated before (Figs. 2B, D, F, arrowheads, compare to 2H). At about 350 h CsNotch and CsDelta 1 and 2 still show a strong expression in the IPGs, whereas transcripts are down-regulated in the remaining cells of the leg (Figs. 2I–K, compare to L).

To summarise, the temporal and spatial expression of the neurogenic genes suggest that they are involved in the selection of the SPGs as well as the specification of cell fates within the SPGs.

Identification of Csnumb and analysis of its expression pattern in the legs

We identified a single numb homologue in the spider C. salei. The initial PCR amplification yielded a fragment of 167 bp, which was extended using 5 rapid amplification of cDNA ends to generate a fragment of 713 bp. A comparison of the deduced amino acid sequence with numb homologues of vertebrates and invertebrates revealed that Csnumb shows the highest similarity to Drosophilidae numb genes (Suppl. Fig. 1).

Although numb is not required for the selection of SOPs in Drosophila, it is expressed in all epithelial cells prior to formation of the SOPs and throughout development. The protein localises to plasma membranes, but it is not asymmetrically distributed in these cells. In the sensory organ lineage, numb is expressed in all cells that require numb function either by de novo expression or asymmetrical distribution during cell division (Rhyu et al., 1994).

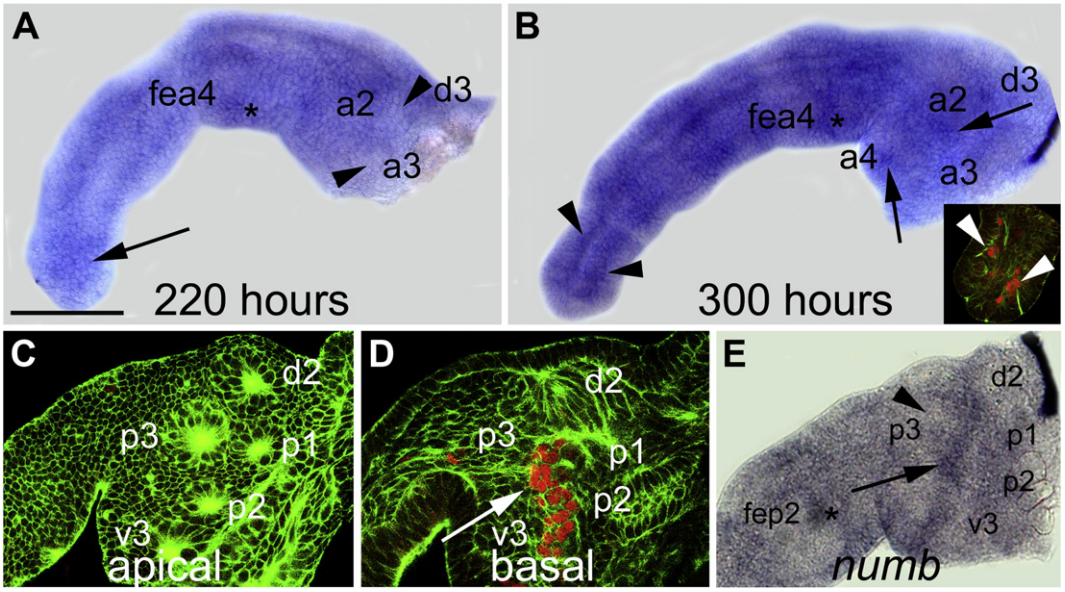

Fig. 3. Csnumb expression during formation of SPGs. Light microscopic images of in situ hybridisations of embryos stained with a DIG-labelled Csnumb probe (A, B, E), confocal laser-scanning micrographs (CLSM) of phalloidin-FITC (green) and anti-Prospero antibody (red) staining (B inset, C, D). (A) Csnumb expression is non-uniform during formation of the SPGs at 220 h (apical view, anterior side). Arrow points to stronger numb expression in the tarsus and asterisk indicates elevated levels of numb transcripts in the femural IPG fea4. The arrowheads point to regions of lower numb expression in the apical part of the coxal IPGs a2 and a3. (B) Basal view of anterior side of leg. Black arrowheads point to elevated levels of Csnumb expression in subepithelial area in the tarsus at 300 h. The inset in panel B shows that in this area neural cells have formed (red, white arrowheads). Higher levels of Csnumb expression correlate to the developing sense organs IPG fea4 (asterisk) and the coxal SPGs a2 and a4 at 300 h (arrows). (C, D) Apical and basal view, respectively, of the posterior coxa at 300 h showing the arrangement of SPGs (bright spots in panel C) and the accumulation of neural precursors in the basal area (red staining in D, arrow). (E) At the same stage, Csnumb transcripts are elevated in the basal area of the coxa (arrow and asterisk), while the expression seems to be lower in the apical part of the IPGs (arrowhead). a2–4, coxal SPGs cxa1–4; d2–3, coxal IPGs cxd2–3; fea4, fep2, femoral IPGs, p1–3, coxal IPGs cxp1–2; v3, coxal IPGs cxv3. Scale bars: (A) 80 μm in A, B, 60 μm in C–E.

Similar to Drosophila, Csnumb is expressed in all epithelial cells throughout embryogenesis (data not shown). In the spider leg, Csnumb expression is non-uniform during formation of the SPGs (Figs. 3A, B, E). Patches of higher expression seem to correspond to subepithelial areas where the neural cells of the SPGs form and sensory neurons from different sensory organs accumulate to form ganglia (Figs. 3A, B, E, arrows; compare to Figs. 3C, D and inset in B). The neuronal cells that delaminate from the 27 tarsal SPGs form several ganglia in the basal area of the tarsus (Fig. 3B, inset, arrowheads). A strong expression of Csnumb is visible in this area (Fig. 3B, arrowheads). Similarly, the neural precursor cells that are generated by the 10 IPGs of the coxa (Fig. 1C) delaminate individually and form tight clusters in the centre of the leg together with neural precursor cells from neighbouring IPGs (Figs. 3C, D). Csnumb is strongly expressed in this basal-central area of the coxa (Fig. 3E, arrow). However, due to the ubiquitous expression of Csnumb in all epithelial cells, we could not determine if neural precursors express slightly elevated levels of the gene prior to delamination.

Analysis of the function of the Notch signalling pathway in sensory organ formation

We analysed the function of the spider Notch and Delta homologues in sensory organ development by RNA interference (see Materials and methods). Embryos were injected before formation of the germ band (about 72 h) and fixed and analysed after 350 h of development, when all characterised SPGs were present in untreated embryos (Fig. 1C). The embryos were stained with the anti-Cupiennius salei-Prospero antibody and phalloidin-FITC. The Prospero antigen is expressed in the neural cells (neurons and glia) of the developing eMSOs and the neural precursors of the IPGs (Stollewerk and Seyfarth, 2008). In contrast to Drosophila, Prospero is not expressed in the sheath cells in the spider. Furthermore, the protein is neither asymmetrically distributed in the eMSO nor in the IPG lineage (Stollewerk and Seyfarth, 2008). Expression is maintained in the neurons until the end of embryogenesis, while it seems to be down-regulated in the glial cells at the time when the sensory neurons develop axons and dendrites. Phalloidin-FITC accumulates in the apical cell processes of the sensory precursor groups (Stollewerk and Seyfarth, 2008). The accessory cells (hair, socket and sheath cells) that arise from the precursors remain attached to each other in the epithelium and phalloidin strongly stains the attachment site, so the developing eMSOs can be visualised with phalloidin-FITC throughout embryogenesis.

After the injection of ds CsNotch RNA 99% of the SPGs on the spider legs were affected. Of the affected SPGs, 71% were missing and the remaining 29% showed the following specific phenotypes (Table 1). In 91% of the present and affected SPGs, the morphology of the sensory precursors was abnormal (Figs. 4A–E). This phenotype is particularly noticeable in the IPGs. In control injected embryos, the IPGs consist of 60 to 80 epithelial cells at 350 h of development. The apical surfaces of these cells are constricted and face each other. Phalloidin strongly stains the apical surfaces of the IPGs due to the presence of actin-rich microvilli, which results in a spot-like staining in horizontal optical sections (Fig. 4B, arrows). In embryos injected with ds CsNotch RNA, the epithelial morphology of the sensory precursors is affected (Figs. 4A, C, compare to B, D, respectively). The apical surfaces are less constricted and do not face each other as in control injected embryos (Fig. 4C, compare to D). Furthermore, in control injected embryos the basal part of the IPGs elongates to form a two-cell wide structure ('stalk') from which individual neural cells delaminate (Fig. 4G, arrows) (Stollewerk and Seyfarth, 2008), but this structure does not form in the affected IPGs (Fig. 4F, arrows).

In addition, the number of accessory cells is reduced in all SPGs that exhibit defects in epithelial morphology and in 49% the neural cells are reduced as well (Figs. 4E, F, H; Table 1). In addition, in 15% of the affected triple-innervated eMSPGs and the affected IPGs the arrangement of the Prospero positive cells is abnormal (Table 1). In control injected embryos the delaminated sensory neurons of the developing triple-innervated eMSOs cluster together and form

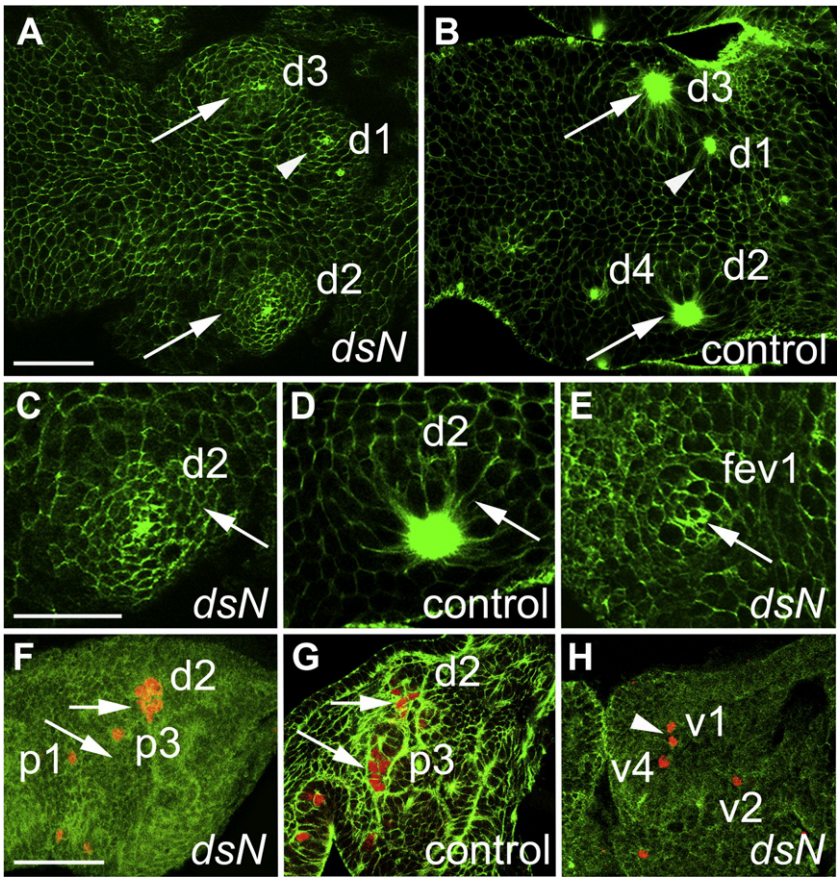

Fig. 4. Notch RNAi phenotype. CLSM images of embryos stained with phalloidin-FITC (green, A–E) and anti-Prospero antibody (red, F–H). (A, B) After Notch RNAi the apical surfaces of the eMSPG cxd1 (arrowheads) and the IPGs cxd2 and cxd3 (arrows) on the dorsal coxa are less constricted and do not face each other as in control injected embryos. (C, D) Higher magnifications of coxal IPGs cxd2 (arrows) in ds Notch RNA and control injected embryos, respectively. (E) Reduction in accessory precursor cells in the femoral IPG fev1. The arrow points to the about 5 cells that are attached to each other which is less than the number of precursor cells that are present when internal joint receptor precursor groups are first visible at mid-embryogenesis. (F, G) The two-cell wide stalk from which the neural precursors delaminate (arrows in panel G) does not form in embryos with reduced Notch signalling (arrows in panel F). (H) Reduction in the number of neural cells. The neurons do not adhere to each other and dendrites are absent (arrowheads). d1–4, coxal SPGs cxd1–4; fev1, femoral IPG; p1, p3, coxal SPGs cxp1, 3; v1, v2, v4, coxal SPGs cxv1, 2, 4. Scale bars: (A) 40 μm in A, B, (C) 25 μm in C–E, (F) 100 μm in F–H.

dendrites at their distal sites, which fasciculate and extend towards the accessory cells of the developing sensory organs. In contrast, in affected eMSPGs which contain more than one neuronal cell, the sensory neurons neither adhere to each other nor develop dendrites (Fig. 4H, arrowhead). Similarly, the delaminated neural precursors of the affected IPGs do not cluster together as in control injected embryos (data not shown).

The expression patterns of the spider Delta homologues suggest that both genes are involved in the development of all MSOs in the legs. By eliminating the function of either Delta 1 or Delta 2, we were able to analyse the effect of Notch signal reduction on sense organ development. After injection of ds Delta 2 RNA, SPG development was more strongly affected than after loss of Delta 1 function (Table 1). Similar to the results from Notch RNA interference (RNAi), more than 90% of the SPGs were affected in ds Delta 2 RNA injected embryos, whereas after Delta 1 RNAi 37% of the SPGs were wildtype. As in ds Notch RNA injected embryos, defects in epithelial morphology and reduction in the number of accessory cells were observed in both ds Delta 1 and 2 RNA injected embryos (Table 1). However, in contrast to Notch RNAi, only 3% and 8% of the affected SPGs show a reduction in neural cells after Delta 1 and 2 RNAi, respectively (Table 1). Instead, there is an increase in the number of neural cells in the developing single- and triple-innervated eMSPGs ranging between 5 and 13 cells (Figs. 5A, C, compare to B, D; Table 1). These cells cluster together and develop dendrites that fasciculate and extend distally (Fig. 5A, arrowhead). In all SPGs that show an increase in the number of neural cells, accessory cells are absent (Fig. 5E, asterisk, compare to G).

In addition, there is a 24% increase in SPGs (Fig. 5K, compare to J; Table 1). Most of the additional SPGs (68%) generate the normal number of 1 or 3 neurons. The neurons are attached to each other and develop dendrites that extend distally as in normal SPGs.

Furthermore, in about half of the affected IPGs the neural cells differentiate prematurely after Delta 1 and 2 RNAi (Delta 1: 54%, Delta 2: 48%; Table 1), while in untreated animals, the IPGs do not differentiate until the second larval stage. After injection of ds Delta 1 and 2 RNA, the Prospero expressing cells develop dendrites and axons (Figs. 5F, I). The dendrites fasciculate and extend apically, while the axons fasciculate and extend proximally. In contrast, in control injected embryos the undifferentiated neural precursors cluster together basally (Fig. 5H). In all IPGs that showed premature differentiation, the epithelial cell groups from which the neural precursors delaminate were absent (Fig. 5E, compare to G).

To summarise, the results show that Notch signalling is involved in multiple aspects of MSO formation in the spider: the maintenance of epithelial morphology, the differentiation state of the developing MSO and the control of the number of neurons in individual SPGs as well as the overall number of SPGs in the leg.

Analysis of numb function in sensory organ formation

During Drosophila sense organ development, Numb is required for preventing Notch signalling in binary cell fate decisions in the neural lineage as well as in the lineage leading to the hair and socket cells. In the neural lineage, Numb is needed for all three cell fate decisions –

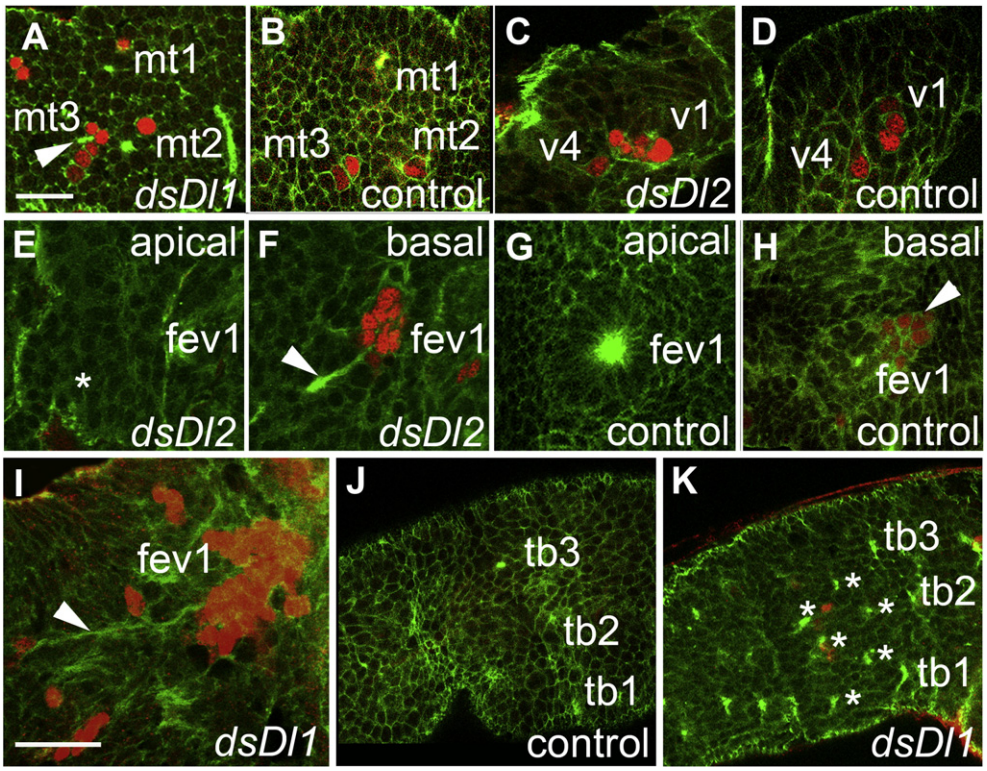

Fig. 5. Delta 1 and Delta 2 RNAi phenotypes. CLSM images of embryos stained with phalloidin-FITC (green) and anti-Prospero antibody (red). (A, C) The number of neural cells is increased after Delta 1 (mt3 in A) and Delta 2 (v1 in panel C) RNAi. The cells cluster together and develop dendrites (arrowhead in panel A). (B, D) Same areas as in panels A and C, respectively, in control injected embryos. (E, F) Apical (1 μm) and basal view (12 μm), respectively, of femoral IPG fev1. Accessory precursors are absent in prematurely differentiating IPGs (asterisk in panel E). Arrowhead in F points to dendrites. (G, H) Apical and basal view, respectively, of same IPG in control injected embryo. Arrowhead in panel H points to clustered neural precursors. (I) Large cluster of neurons in the femoral IPG fev1 after Delta 1 RNAi. Arrowhead points to axon fascicle. (J) Pattern of eMSPGs on the tibia of a control injected embryo. (K) Additional SPGs (asterisks) on the tibia after Delta 1 RNAi. fev1, femoral IPG; mt1–3, metatarsal eMSPGs mta1–3; tb1–3, tibial eMSPGs tba1–3. Scale bars: (A) 20 μm in A–H, (I) 30 μm in I, 40 μm in J, K.

first in the neural precursor pIIb, then pIIIb and finally in the neuron – whereas it is only required in the hair cell in the accessory lineage. In numb mutants, the number of sensory neurons is reduced and in the most severe phenotypes the sensory lineage consists exclusively of socket cells.

After injection of ds Csnumb RNA, 98% of the SPGs were affected in the spider legs. 63% of the affected SPGs were either missing or not traceable with the applied markers (Table 1). Similar to Drosophila, the number of neural precursors and neuronal cells is reduced in 55% of the affected SPGs (Table 1; Fig. 6A, asterisk, G, compare to D). In 2% of the affected eMSPGs the number of Prospero positive cells is increased (Fig. 6A, arrowhead). In addition, the morphology of the sensory precursor cells is abnormal in half of the affected SPGs (Figs. 6E, F). They have a rounded appearance and lack the typical columnar shape of epithelial cells. Apical cell processes are absent and the sensory precursors form cell clots (Figs. 6E–G). There is a higher accumulation of F-actin in the cell cortices as compared to the control (Fig. 6F, compare to Fig. 5G).

Furthermore, we found that the delamination of the neural precursors is affected in ds Numb RNA injected embryos. We only documented this phenotype in the IPGs as differences in delamination pattern are difficult to detect in the eMSPGs. This is because the neural marker Prospero is already expressed in the neural precursors of the eMSPGs before they delaminate and occupy a basal position. Therefore we could not determine if the apical expression of Prospero is due to a defect in delamination or rather reflects a delay in development due to the injection procedure. In contrast, Prospero is not expressed in the apical epithelial cells throughout IPG development, but in the basal part of the 2-cell wide stalk and in the neural precursor cells that have delaminated from the stalk (Fig. 6B, arrow). Although the stalk forms after numb RNAi, in 65% of the affected IPGs the neural precursors do not delaminate from the stalk but remain attached to it like a bunch of grapes (Fig. 6A, arrow; compare to Fig. 6B, arrow). In control embryos the delaminated neural precursors of adjacent IPGs intermingle in basal areas of the leg and form tight clusters (Fig. 6D). These clusters do not form in ds numb RNA injected embryos, providing further evidence for a delamination defect (Fig. 6C).

To summarise, Csnumb seems to be required in the accessory and the neural cells of the developing MSOs of the spider. The observed RNAi phenotypes are only partially comparable to Drosophila numb mutants and therefore it is not clear if sensory organ development is affected in the same way in both species.

Are numb and members of the Notch signalling pathway involved in binary cell fate decisions in spider sense organs?

In Drosophila, elimination of Notch signalling leads to the transformation of pIIa – the precursor of the hair and socket cells – to the neural precursor pIIb. In addition, Notch is required in the sheath cell for the final binary cell fate decision between neuron and sheath cell in the neural lineage. Thus in mutants of the Notch signalling pathway there is an overproduction of sensory neurons at the expense of accessory cells. Numb mutants exhibit the opposite phenotype: pIIb is transformed into pIIa. In severe phenotypes, pIIa exclusively generates socket cells at the expense of hair cells, since numb is required in the hair cell as well.

Our results show that reducing Notch signalling by eliminating either Delta1 or Delta 2 function results in an overproduction of sensory neurons in the spider. In the affected eMSPGs accessory cells are absent, indicating that the additional neurons are generated at the expense of accessory cells. This assumption is supported by analysis of the mitotic pattern after Delta RNAi. During the development of the

eMSPGs the initial group sizes of 6–9 cells increase by cell proliferation to 12 plus 2 and 12 plus 4 neural cells (see Fig. 1B). One mitotic cell which is located in the epithelium is associated with each eMSPG throughout development. In addition, one neural precursor divides after delamination in the developing triple-innervated eMSOs (see Fig. 1B). Overall the number of mitotic cells in the leg is reduced after injection of ds Delta1 and Delta 2 RNA, owing to missing SPGs and a reduction of sensory precursor cells in some of the affected SPGs (Table 1). However, as in control embryos, one mitotic cell and in some cases one mitotic neural precursor is associated with eMSPGs that show an increase in neural cells, indicating that the additional cells are not generated by hyperproliferation of sensory precursor cells (Suppl. Fig. 2).

In the absence of markers for hair, socket and sheath cells (accessory cells) we were unable to analyse if Csnumb plays a role in binary cell fate decisions in the developing sense organs of the spider.

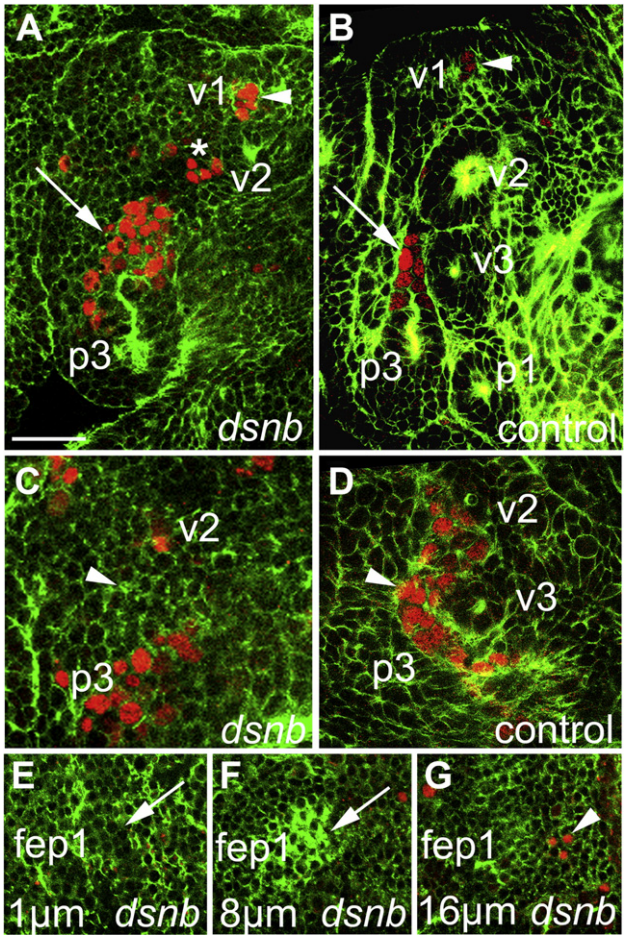

Fig. 6. Csnumb RNAi phenotype. CLSM images of embryos stained with phalloidin-FITC (green) and anti-Prospero antibody (red). (A) The number of neural precursors is reduced (v2, asterisk) after numb RNAi. In 2% of the affected SPGs neural cells are increased (v1, 5 Prospero positive cells, arrowhead). Delamination is affected and neural precursors are attached to the stalk (arrow). Instead of losing contact to the precursor group and segregating towards the centre of the leg, the neural cells remain attached to the IPG p3 and therefore there seem to be more neural precursors as in control injected embryos. (B) Same area in control injected embryo. Arrow points to stalk. Arrowhead points to neural cells of v1 (3 Prospero positive cells). (C) Neural precursors of adjacent IPGs do not intermingle (arrowhead) after numb RNAi. (D) Same area in control injected embryo. Arrowhead points to neural precursors that intermingle in the area between 2 IPGs. (E–G) Optical sections of 3 different apico-basal levels of femoral IPG fep1 after numb RNAi. Apical cell processes are absent (arrow in E, apico-basal level: 1 µm) and the cells form cell clots (arrow in F, apico-basal level: 8 µm). Arrow points to reduced number of neural precursors in panel G (apico-basal level: 16 µm). fep1, femoral IPG; p1, p3, coxal IPGs cxp1, 3; v1–v3, coxal IPGs cxv1–3. Scale bars: (A) 50 µm in A, B, E–G, 25 µm in C, D.

Table 2
Effect of Delta (Delta 1 and 2), Notch and numb RNAi on apoptosis of neural cells in the developing MSOs of the spider

Treatment	IPGs		eMSPGs	
	Mean no. of apoptotic cells (N_c)	p-value for N_c for treatment = N_c of control	mean no. of apoptotic cells (N_c)	p-value for N_c for treatment = N_c of control
Delta RNAi	0.67	0.037	0.14	0.20
Notch RNAi	2.67	0.0063[a]	0.63	3.02×10^{-10}[b]
numb RNAi	12.54	1.17×10^{-9}[b]	0.77	6.66×10^{-11}[b]
Control	0.20	–	0.08	–

The overall tests confirmed that the RNAi treatments had a significant effect on apoptotic cells counts for both IPGs and eMSPGs. (Kruskal–Wallis rank sum test; df = 3 for both tests. eMSPGs, $\chi^2 = 73.71$, $p = 6.9\times10^{-16}$. IPGs, $\chi^2 = 57.05$, $p = 2.5\times10^{-12}$). Individual tests show a significant effect of RNAi treatment with numb on apoptosis in all SPGs, and a significant effect of Notch on eMSPGs. The effect of Notch RNAi on IPGs was only marginally significant. Delta RNAi treatment had no effect on either type of organ (see Materials and methods for further details).

[a] Indicates comparisons significant at the 0.05 level following correction.

[b] Indicates comparisons significant at the 0.01 level following correction.

However, as described above, mutations in Drosophila that affect the members of the Notch signalling pathway and Numb lead to cell fate transformations, rather than differentiation defects and cell death. Thus, if the spider numb homologue were involved in binary cell fate decisions in a similar way, we would not expect an increase in neural cell death.

First we analysed the distribution of neural apoptotic cells in the peripheral nervous system of control animals to see if programmed cell death is an integral part of sense organ development in the spider. (In some eMSO lineages of Drosophila one daughter cell of the tertiary neural precursor – a glial cell – undergoes apoptosis during normal development (Fichelson and Gho, 2003). The eMSPGs and the IPGs were analysed separately because of the considerable difference in cell group sizes. The mean number of neural apoptotic cells in the eMSPGs is 0.08 and 0.20 in the IPGs, indicating that apoptosis is not part of the normal developmental programme (Table 2; Figs. 7A, B). After injection of either Delta 1 or Delta 2 ds RNA, apoptosis is neither significantly increased in the eMSPGs nor in the IPGs (Table 2; Figs. 7A, D). Elimination of Notch function by injection of ds Notch RNA resulted in considerable increase in neural apoptotic cells in the eMSPGs and a marginal increase in the IPGs, while numb RNAi substantially enhances the number of neural apoptotic cells both in eMSPGs and IPGs (Table 2; Figs. 7A, C, E).

These data suggest that Csnumb is primarily required for the correct differentiation and/or survival of neural cells in the developing MSOs, while Notch signalling is involved in cell fate decisions as well as differentiation of the sensory precursors.

Discussion

We show here that Notch signalling and Csnumb function are required for the development of MSOs in the spider C. salei. In the following paragraphs we discuss the role of Notch, Delta 1, Delta 2 and numb at the different stages of MSO generation in the spider.

Step 1 — Selection of sensory precursor groups

The first step in sensory organ formation is the selection of sensory precursors from a competent epithelium (Hartenstein, 2005). We have shown recently that both spider achaete–scute homologues are required for this process (Stollewerk and Seyfarth, 2008). In contrast to Drosophila, the genes are not expressed in well-defined proneural clusters, but at low levels in the whole leg epithelium. Both genes are up-regulated in epithelial cells that are specified as sensory precursors. We show here that the expression of the spider Notch and Delta homologues coincides with the expression domains of the

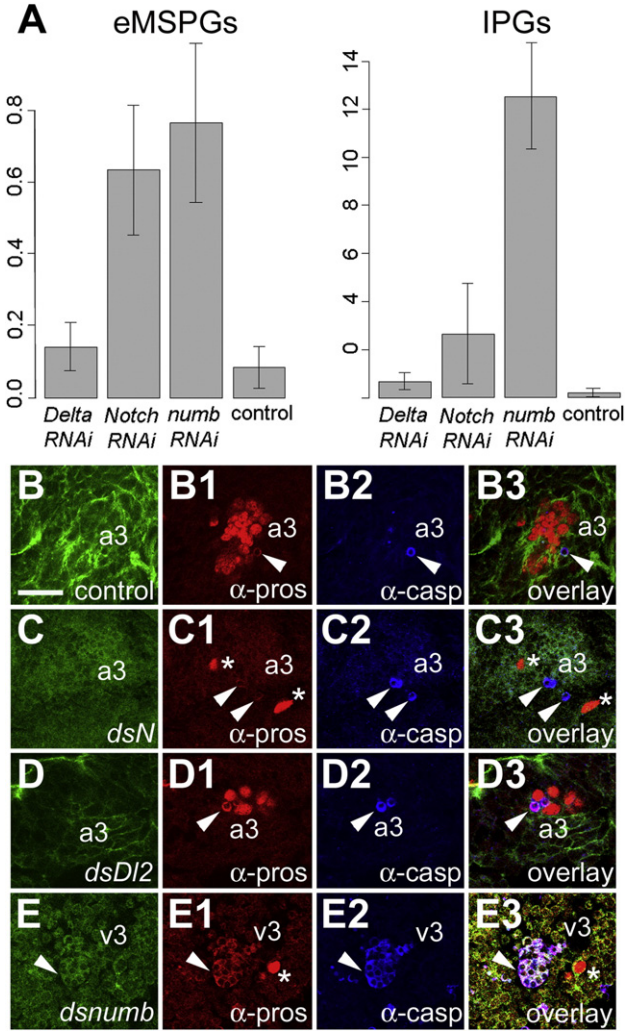

Fig. 7. Effect of Delta (Delta 1 and 2), Notch and numb RNAi on apoptosis of neural cells in the developing MSOs of the spider. (A) See Table 2 legend for details. (B–E) CLSM images of embryos stained with phalloidin-FITC (green), anti-Prospero antibody (red) and anti-Caspase 3 antibody (blue); basal optical images. Arrowheads point to apoptotic neural cells. Asterisks indicate Prospero positive cells that do not co-express the apoptotic marker. (B–B3) The control coxal IPG contains 1 apoptotic neural cell. (C–C3) Apoptotic neural cells are only marginally increased in IPGs after Notch RNAi. (D–D3) No significant increase of apoptotic neural cells after Delta 1 and 2 RNAi. (E–E3) Significant increase of neural apoptotic cells after numb RNAi. a3, coxal IPG cxa3; v3, coxal IPG cxv3. Scale bar: (B) 20 μm.

achaete–scute homologues. However, while the expression of the achaete–scute homologues rapidly decreases in the selected sensory precursors, transcripts of the neurogenic genes accumulate in the SPGs throughout their development as in Drosophila. This is especially obvious in the IPGs, which do not differentiate until the second larval stage and continuously express the neurogenic genes.

Our functional data show that a reduction in Notch signalling by eliminating Delta 1 or Delta 2 function leads to the selection of additional SPGs. These results imply that Notch signalling prevents epithelial cells adjacent to SPGs from adopting the same fate by lateral inhibition (Fig. 8). We have shown previously that lateral inhibition mediated by Notch signalling restricts the number of neural precursors in the central nervous system of the spider (Stollewerk, 2002).

As in the spider, the Drosophila achaete–scute homologues initially confer the competence to enter the sensory organ pathway upon groups of epithelial cells, the proneural clusters, which arise at predetermined positions. However, in contrast to Cupiennius, these clusters are later refined to one or a few cells, the future SOPs, by lateral inhibition in Drosophila. Thus, Notch signalling is active within the proneural clusters in Drosophila and leads to the selection of individual SOPs, whereas in the spider all cells with elevated levels of the achaete–scute homologues become sensory precursors (Fig. 8). These data lead to a model where ubiquitous Notch signalling generally reduces proneural gene expression in the leg epithelium of the spider to levels too low to initiate sensory precursor development. At predetermined positions Notch signalling is inhibited in cells that have elevated levels of Delta, which in turn de-represses proneural gene expression and facilitates sensory precursor formation. This model is in line with recently published data on the pre-patterning of the positions of sensory bristles by Delta and Hairy in the Drosophila leg. Delta and Hairy act together to set up achaete expression in longitudinal stripes from which the small leg bristles arise. Notch signalling is not active in the achaete expressing stripes, which co-express high levels of Delta (Joshi et al., 2006).

Step 1A — Maintenance of epithelial morphology and control of differentiation state

In Drosophila, the second step in MSO development is the decision between neural and hair/socket precursor cell fate. Our results show that Notch signalling is required for an intermediate stage between sensory organ precursor selection and precursor specification in the developing MSOs in the spider. This stage does not have a counterpart in eMSO formation in Drosophila.

Our functional data show that Notch signalling is required for maintaining the epithelial morphology of the SPGs (Fig. 8). In the absence of Notch signalling the strong adhesion between the apical

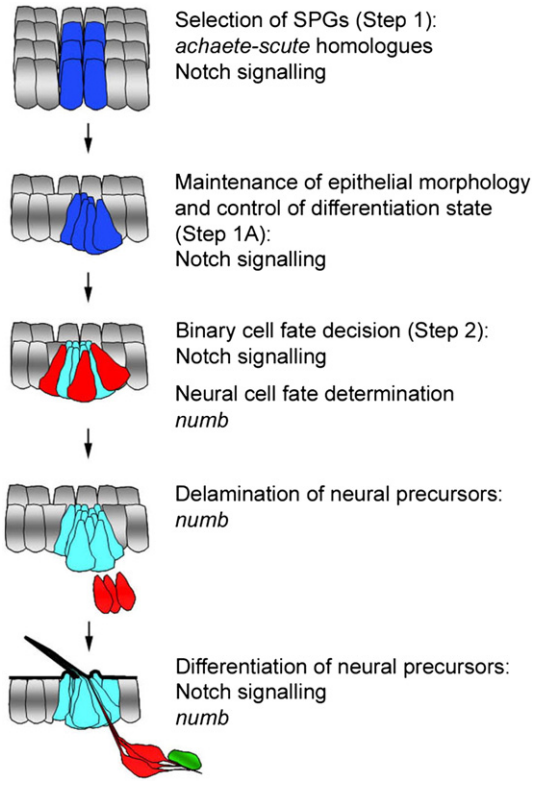

Fig. 8. The role of Notch signalling and numb function at the different stages of MSO generation in the spider. The scheme represents the development of triple-innervated eMSOs in the spider. For simplification the scheme does not show the cell proliferation of the SPG and the division of one of the neural precursor cells after delamination (see Fig. 1B). Grey, leg epithelium; blue, sensory precursors; light-blue, accessory precursors (accessory cells); red, neural precursors (neurons); green, glial cell. See text for further details.

cell processes of the sensory precursor cells is disrupted. In addition, in the IPGs the basal stalk-like structure from which the neural precursors delaminate does not form. We assume that these morphological defects eventually lead to the loss of precursor cells and consequently to a reduction in accessory and neural cells and even to a complete loss of SPGs in severe phenotypes, as observed in Notch and Delta RNAi embryos.

This assumption is supported by recent data on Notch signalling in both vertebrates and invertebrates. Inactivation of the Notch effector genes Hes 1, 3 and 5 in mice results in reduction of tight and adherens junctions at the apical end feed of neural stem cells and loss of these cells from the spinal cord neuroepithelium (Hatakeyama et al., 2004). In Drosophila Notch signalling seems to be required in the olfactory sensory precursors in a similar way to the spider (Ray and Rodrigues, 1995; Reddy et al., 1997; Sen et al., 2003). The first step in olfactory sense organ development is the specification of a founder cell, which is followed by the specification of two to three adjacent cells. Together these cells form the 'presensillum cluster', which divides as a group to give rise to the cells of the olfactory sensillum. Rodrigues and co-workers (Reddy et al., 1997) showed that the founder cells in Notch mutant antennae form but are unable to maintain presensillum clusters. Consequently, olfactory sense organs are missing in these flies.

Interestingly, we observed the opposite phenotype in ds numb RNA injected embryos. In contrast to Notch loss of function, adhesion is increased among the sensory precursors after numb RNAi. The sensory precursors retract their apical processes but their cell bodies remain attached to each other and form cell clots. The increased adhesion seems to prevent the neural precursors from delaminating from the precursor groups. We assume that this defect has an impact on the correct differentiation of the neural cells and eventually leads to the removal of these cells by apoptosis. This assumption is supported by the considerable increase in apoptosis in cells that express the neural cell fate determinant Prospero in all SPGs after numb RNAi. Thus, although Notch signalling and Numb affect different processes of sense organ development, functional disruption of both genes seems to lead to defects in the differentiation of neural cells and to an increase in apoptosis.

The opposite functions of Notch and Numb in sense organ formation suggest that numb facilitates the delamination of neural precursors by inhibiting Notch signalling in sensory precursor cells that have entered the neural pathway (Fig. 8). In the Drosophila sensory organ lineage, numb function has only been analysed in the context of asymmetric cell division and binary cell fate decision, rather than morphological changes. It is obvious that inhibition of Notch signalling by Numb in the neural precursors pIIb and pIIIb does not result in the delamination of these cells, since they remain in the epithelium. However, the progeny of pIIIb – a neuron and a glial cell – inherit numb in two consecutive cell divisions and occupy subepithelial positions. Numb might be involved in the segregation of these cells but this has not yet been analysed.

Interestingly, some aspects of the numb/numblike loss of function phenotype in vertebrates resemble the numb RNAi phenotype in the spider. First of all, neural progenitors in the ventricular zone of the spinal cord retract their apical end feet in numb/numblike knockout mice and the affected neural progenitors form cell clots ('rosettes'; Rasin et al., 2007). The morphology of these rosettes is comparable to the cell clots formed by the sensory precursors in the spider after numb RNAi. Both in the mouse and in the spider the precursor cells lose their epithelial morphology and the arrangement of the actin cytoskeleton is disturbed.

Furthermore, it has been shown in another study on numb function in the forebrain that Cajal-Retzius neurons are displaced from their normal location in the outer layer of the neocortex to ectopic sites in numb/numblike knockout mice (Li et al., 2003). Some of them are located close to the apical surface, indicating that the delamination of neural precursors is affected. In addition, the number of neurons is reduced by half, which is accompanied by an increase in neuronal apoptosis. Our data suggest that Notch and numb might play a similar role in the delamination of neural precursors in the developing vertebrate central nervous system as in the peripheral nervous system of the spider.

Furthermore the RNAi data show that the maintenance of the epithelial morphology has an impact on the differentiation state of the sensory precursors in the spider.

Reducing Notch signalling results in premature differentiation of neural precursors in the developing internal joint receptors, which do not differentiate until the second larval stage in untreated animals. The prematurely generated sensory neurons develop dendrites which fasciculate and extend aberrantly towards the leg surface. Furthermore, in 15% of the triple-innervated eMSPGs and IPGs which are devoid of Notch function, the neuronal cells do not cluster together as in the control. In these eMSPGs the neurons do not develop dendrites and apoptosis is increased. These data suggest that Notch keeps the sensory precursors in an undifferentiated state and that the time point of entering the neural pathway and delamination is crucial for the correct differentiation of the sense organs (Fig. 8). Our results are supported by recent publications on vertebrate neurogenesis which show that Notch signalling is required for maintaining neural progenitor cells in an undifferentiated state (Dooley et al., 2003; Hatakeyama et al., 2004; Toriya et al., 2006). Furthermore, the direct involvement of Notch in sensory neuron differentiation has been demonstrated recently in the olfactory sense organs of Drosophila, where Notch activity is required for the correct axonal projections of olfactory neurons to the antennal lobe (Endo et al., 2007).

Step 2 — Binary cell fate decision between neural and hair/socket precursor cells

In the Drosophila sensory organ precursor lineage, loss of Notch signalling leads to the transformation of the hair/socket precursor pIIa to the neural precursor pIIb, while loss of numb leads to a pIIb to pIIa conversion. Notch and Numb are again required for binary cell fate decisions at the next level between the neuron and the sheath cell and hair and socket cells. In Notch mutants complete transformations result in all cells of the sensory organ adopting the neuronal fate, while completely transformed numb mutant sense organs consist exclusively of socket cells (Hartenstein and Posakony, 1990; Uemura et al., 1989).

If Notch signaling is not completely disrupted but rather reduced by either removing Delta1 or Delta2 function, the sensory precursors are still sufficiently attached to each other so that sensory organ development can proceed to the next step. In this case, the reduction of Notch signalling leads to an increase in neural cells in the eMSPGs of the spider. We assume that the increase is due to changes in the cell fate of sensory precursors which give rise to accessory cells in control animals, since these cells are absent in the affected eMSPGs and the additional neural cells do not arise by hyperproliferation of sensory precursors. In a similar way, loss of Notch function after formation of the polyclonal presensillum cluster leads to an increase in neural cells (neurons and glia) in the developing olfactory sense organs of Drosophila (Sen et al., 2003). It is interesting to note that binary cell fate decisions can occur between sensory precursors that are not lineage related.

Our data do not allow us to resolve whether numb is involved in binary cell fate decisions in the spider MSOs, since markers for the accessory cells are missing and numb RNAi spiders do not survive until the second larval stage, when the first MSOs appear in the cuticle (Stollewerk and Seyfarth, 2008). However, the reduction in neural cells and the defects in delamination of the

neural cells suggest that numb does play a role in neural cell fate determination in MSO development in the spider (Fig. 8). We speculate that the Notch signalling to Numb ratio regulates the expression of the neural cell fate determinant Prospero. Both genes are expressed in all sensory precursor cells, however, slightly elevated levels of numb in the basal cells of the developing sense organ could reduce Notch signalling and shift the Notch to Numb ratio within these cells. This would allow individual precursors to express the neural cell fate determinant Prospero and delaminate from the precursor group. This model is supported by the fact that a reduction in Notch signalling leads to additional cells expressing Prospero in the spider SPGs, while the absence of numb function results in a reduction of neural cells. A correlation between Notch signalling and Prospero expression has been demonstrated in the adult MSO lineage of Drosophila. In the numb expressing pII precursor cell, prospero is expressed de novo, while Notch suppresses prospero expression in the pIIa precursor (Reddy and Rodrigues, 1999).

In addition, numb seems to be required for the correct differentiation and/or survival of sensory neural cells, since these cells partially form after numb RNAi but undergo apoptosis. These data are supported by publications on the analysis of numb/numblike function in the peripheral nervous system of vertebrates that show differentiation abnormalities in sensory ganglia which are not accompanied by defects in sensory precursor formation (Huang et al., 2005; Zilian et al., 2001).

Evolutionary comparison

If we assume that sensory precursor formation in the spider represents the ancestral mode of development, we can deduce that the SPGs in the spider are homologous to the proneural clusters of the corresponding sense organ types in Drosophila. All cells that are competent to adopt the sensory precursor fate at a given time enter the sensory organ pathway in the spider. In Drosophila, this part of the developmental programme has changed by adding a further step. Notch signalling is not only required for determining the size of the proneural clusters but also for singling out individual (or several) SOPs in Drosophila. In the spider, Notch signalling is furthermore required for maintaining the epithelial morphology and thus the undifferentiated state of the sensory precursors. This part of the developmental programme has been maintained in the Drosophila sense organs that arise from mixed lineages (e.g. olfactory sense organs), but does not have a counterpart in the remaining developing sense organs.

Since the neural cell fate determinant Prospero is not asymmetrically distributed in the spider PNS (Stollewerk and Seyfarth, 2008), we suggest that cell fate determinants are expressed de novo in the sensory precursors, at least in the neural lineages. The expression of the cell fate determinants seems to be regulated by the Notch signalling to Numb ratio within the sensory precursors. Drosophila sensory cell type identity is determined by lineage (asymmetric distribution) as well as de novo expression of cell fate determinants, suggesting that one part of the ancestral cell identity programme is conserved in Drosophila, while an extra step has been added. Further analysis will reveal how the different cell fates of the accessory cells of the spider MSOs are determined.

Acknowledgments

This work was supported by a Heisenberg Fellowship from the German Research Foundation to AS. The experiments were partially performed in Pat Simpson's laboratory at the Department of Zoology, University of Cambridge, UK and in Gerd Technau's laboratories at the Department of Genetics, University of Mainz. We are grateful to Pat Simpson and Gerd Technau for providing lab space and for stimulating discussions. We thank Ernst-August Seyfarth for providing fertilised C. salei females and cocoons.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2008.12.004.

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