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A homolog of the hydrolase *Notum* is expressed during segmentation and appendage formation in the Central American hunting spider *Cupiennius salei*

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Abstract The hydrolase Notum (also known as Wingful) has been implicated in shaping the distribution gradient of the morphogen Wingless in *Drosophila* by modifying the Wingless-binding heparan sulfate proteoglycan (HSPG) core glypicans Dally and Dally-like. Here we report on the expression of a *Notum* homolog during the embryonic development of the spider *Cupiennius salei*. *Notum* is expressed in two to three stripes in the posterior region of the germband where new segments are formed. At this location no *Wingless* expression is present, suggesting that Notum may be involved in the regulation of another HSPG-binding morphogen, possibly Hedgehog. In older segments, however, expression of *Notum* and *Wingless* roughly coincides. In the appendages, *Notum* is expressed in ventral mesodermal cells, directly adjacent to the *Wingless* expressing ectodermal cells. This could indicate a role for the mesoderm in regulating morphogen gradient formation in the ectoderm.

Introduction

Morphogen gradients play an important role in pattern formation during animal development [reviewed in Gonzalez-Gaitan (2003)]. One example is the gradient formed by the morphogen Wingless (Wg) in the fruitfly *Drosophila melanogaster*. Wg plays a role in segmentation, wing and leg development, and several other processes (e.g., Kubota et al. 2003; Siegfried and Perrimon 1994). In most of these contexts, Wg is secreted by a small number of cells and is bound immediately to the surface of these cells by heparin sulfate proteoglycans (HSPGs) present on the cell membrane (Gonzalez et al. 1991; van den Heuvel et al. 1989; Reichsman et al. 1996; Binari et al.

1997; Tsuda et al. 1999; Lin and Perrimon 1999; Baeg et al. 2001). *Drosophila* has four different kinds of HSPG core glypicans, two of which, Dally and Dally-like (Dlp), have recently been subject to intensive studies (Desbordes and Sanson 2003; Franch-Marro et al. 2005; Han et al. 2004, 2005; Kirkpatrick et al. 2004; Kreuger et al. 2004; Lin and Perrimon 1999; Tsuda et al. 1999). Novel results indicate that Wg proteins bound to Dally are presented to Wg receptors present on the same cell, whereas Wg bound to Dlp is transported to neighboring cells (Franch-Marro et al. 2005; Han et al. 2005). Binding of Wg to HSPGs is therefore essential for both Wg signaling and Wg gradient formation. The hydrolase Notum (also known as Wingful) is an enzyme that appears to interfere with both of these processes by cleaving the HSPG core proteins, thus removing bound Wg protein from the cell surface and making it unavailable for signaling and gradient formation (Gerlitz and Basler 2002; Giraldez et al. 2002; Kirkpatrick et al. 2004; Kreuger et al. 2004). By controlling the level of available Wg, Notum has an important influence on Wg signal transduction and on the shape of the Wg gradient.

Homologs of *Wingless* from *Drosophila* have been isolated from a number of arthropod species (e.g., Nulsen and Nagy 1999; Damen 2002; Hughes and Kaufman 2002; Janssen et al. 2004; Prpic 2004). Although the available expression patterns have already suggested that the role of *Wingless* may be conserved among all arthropods, the existence of a Wg gradient has yet to be demonstrated in arthropods other than *Drosophila*. Among the prerequisites for a conserved mode of Wg gradient formation would be the presence of factors involved in distributing Wg (e.g., Dlp) or shaping its distribution gradient (e.g., Notum). Comparative studies of such factors in other arthropod species, however, are lacking. As a first step, we report here on the cloning and expression analysis of a *Notum* homolog in the spider *Cupiennius salei*.

Results and discussion

We have searched publicly accessible sequence databases (at <http://www.ncbi.nlm.nih.gov/BLAST/> and at

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<http://www.bioinformatics.ksu.edu/BeetleBase/index.html>) for sequences similar to *Drosophila melanogaster* Notum using the tblastx algorithm (Altschul et al. 1997). A Notum homolog is present in the genome of *Drosophila pseudoobscura*, a species closely related to *D. melanogaster*. Sequences similar to Notum are also present in the genomic sequence of the insects *Anopheles gambiae* (mosquito), *Apis mellifera* (honeybee), and *Tribolium castaneum* (flour beetle). No Notum homolog has been found in the available genomic sequence from another insect, *Bombyx mori* (silkworm), possibly because the *Bombyx* genome sequence is still incomplete. Notum-like sequences were also found in the genomes of several vertebrates including human, chicken, mouse and rat. The fugu genome apparently contains four Notum-like genes, but no Notum-like sequence has been found in the sequence available from zebrafish. Notably, the two completely sequenced genomes of the nematode worms *Caenorhabditis elegans* and *C. briggsae* lack Notum-like genes, indicating gene loss in the Nematoda. Nonanimal proteins with similarity to Notum are plant pectin acetyltransferases (Giraldez et al. 2002; Gerlitz and Basler 2002). We isolated a homolog of Notum from the spider *Cupiennius salei* using a set of degenerate primers (Notum fw: TNG CNG GNW SNW SNG CNG GNG G; Notum bw: GNG CYT CRT CRA ANA RCC AYT G). A phylogenetic analysis (Fig. 1) using all available arthropod Notum protein sequences (including the sequence from

Cupiennius), the vertebrate Notum-like sequences, and a selection of plant pectin acetyltransferases, shows that the arthropod Notum proteins and the vertebrate Notum-like sequences form two separate groupings with significant statistical support. The spider sequence is included in the group containing the insect Notum homologs, and the corresponding gene is therefore designated as *Cs-Notum* (GenBank accession number: AJ871012).

The spatio-temporal expression profile of *Cs-Notum* was then examined by whole-mount in situ hybridization. The expression level of *Cs-Notum* is extremely low and detection of specific signals was achieved only after staining for at least 20 h (the normal staining interval for strongly expressed genes like *Distal-less* or *dachshund* in *Cupiennius* is 1–2 h). Aside from expression in the stomodaeum (not shown), *Cs-Notum* mRNA is expressed during segment formation. Expression is seen in two or three discontinuous stripes at the posterior end in the region where new segments are formed (Fig. 2a–c, arrowheads). As it matures, each segment loses *Cs-Notum* expression, but expression is initiated again in older segments in the dorsal portion of the germband anterior to the parasegment borders (Fig. 2c, arrows). This expression pattern suggests a role of Notum in segment formation and segment border establishment. In the dorsal part of the older segments Notum may interact with Wg signaling, because *Cs-Wingless* is also expressed there (Damen 2002; Fig. 2d). However, Notum is unlikely to interact with Wg signaling in newly forming segments, because *Cs-Wingless* is not expressed there, but is expressed at the very posterior end of the germband (Damen 2002; Fig. 2d). Therefore, it is likely that in forming segments Notum interacts with another HSPG-binding morphogen. Among others, the morphogen Hedgehog has been shown to bind to HSPGs in *Drosophila* (The et al. 1999; Desbordes and Sanson 2003; Han et al. 2004). So far, we have not been able to isolate a *hedgehog* homolog from *Cupiennius*, but data from another chelicerate show that *hedgehog* is expressed in a segmental pattern in newly forming segments (Simonnet et al. 2004). We therefore speculate that the segmental *Cs-Notum* expression in forming segments may indicate an interaction of Notum with Hedgehog signaling in *Cupiennius*.

Cs-Notum is expressed at low level in all prosomal appendages (Fig. 3). Expression of *Cs-Notum* is restricted to the ventral side of the appendages, suggesting a possible interaction of Notum with Wg gradient formation. However, *Cs-Notum* is expressed in the ventral mesoderm (Fig. 3c–f; arrow), while *Cs-wg* is expressed in the adjacent ventral ectoderm (Prpic et al. 2003). Thus, if the influence of Notum on Wg protein availability is conserved in the appendages of *Cupiennius*, then our data suggest that HSPGs and bound Wg are removed from the surface of Wg expressing ventral leg cells by Notum enzymes secreted from adjacent mesodermal cells. This could represent a fundamental difference between leg development in *Cupiennius* and leg imaginal disc development in *Drosophila*, where an influence of the mesoderm on pattern formation in the ectoderm has not yet been described.

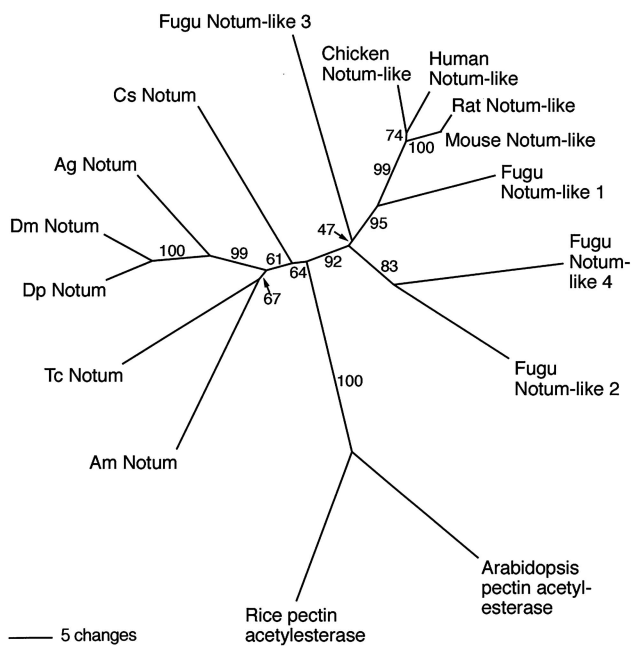


Fig. 1 Phylogenetic analysis of arthropod Notum protein sequences. The analysis included partial protein sequences from *Tribolium castaneum* (Tc), *Apis mellifera* (Am), *Drosophila melanogaster* and *D. pseudoobscura* (Dm, Dp), *Anopheles gambiae* (Ag), *Cupiennius salei* (Cs), fugu, human, mouse, rat, chicken and two selected plant pectin acetyltransferases (from *Arabidopsis* and rice). Shown is the unrooted puzzle tree computed from 1000 intermediate trees produced with Puzzle (implemented in PAUP 4.0b10) (Strimmer and von Haeseler 1996; Swofford 2002)

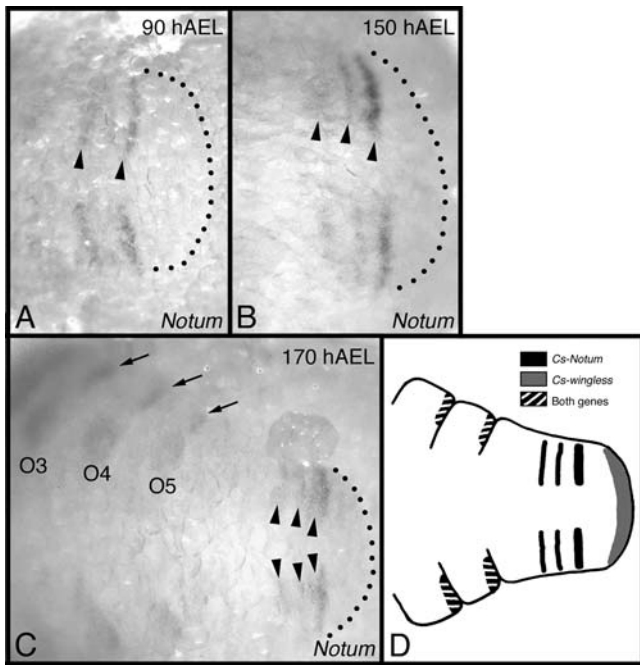


Fig. 2 Expression of *Cs-Notum* during germband segmentation. **A** Posterior end of an embryo at approximately 90 h after egg laying (AEL). The very posterior end of the germband is indicated by the dotted line in all panels. *Cs-Notum* is expressed in two stripes (arrowheads) in the area where new segments are formed. **B** Posterior end of an embryo at approximately 150 h AEL. Three stripes of *Cs-Notum* expression can be seen (arrowheads). **C** Posterior end of an embryo at approx. 170 h AEL. *Cs-Notum* transcripts are present in three stripes in the area where the new segments form (arrowheads). Older segments have expression in their dorsal portion (arrows). **D** Simplified schematic drawing of the posterior end of the germband combining the expression patterns of *Cs-Notum* (black) and *Cs-Wingless* (gray); overlap of expression indicated by hatching. Please note that coexpression is inferred only from the fact that both genes are expressed anterior to the parasegment groove. Double-labeling was not possible due to technical problems with the extremely low level of *Cs-Notum* expression. All embryos are shown in ventral aspect, with anterior pointing to the left. Abbreviations: O3–O5, opisthosomal segment 2–5

The presence of *Notum*-like genes in the genomes of diverse arthropod species indicates an important, evolutionarily conserved role of this gene in the arthropods. The expression data in *Cupiennius* give support for an interaction of *Notum* with *Wg* signaling, since in older segments and in the developing appendages both genes are expressed in overlapping patterns or in directly adjacent tissues. However, the expression pattern of *Notum* in the newly forming segments is unanticipated, since no *Wingless* expression is found there. This may suggest the interaction of *Notum* with other HSPG-binding morphogens (e.g., Hedgehog).

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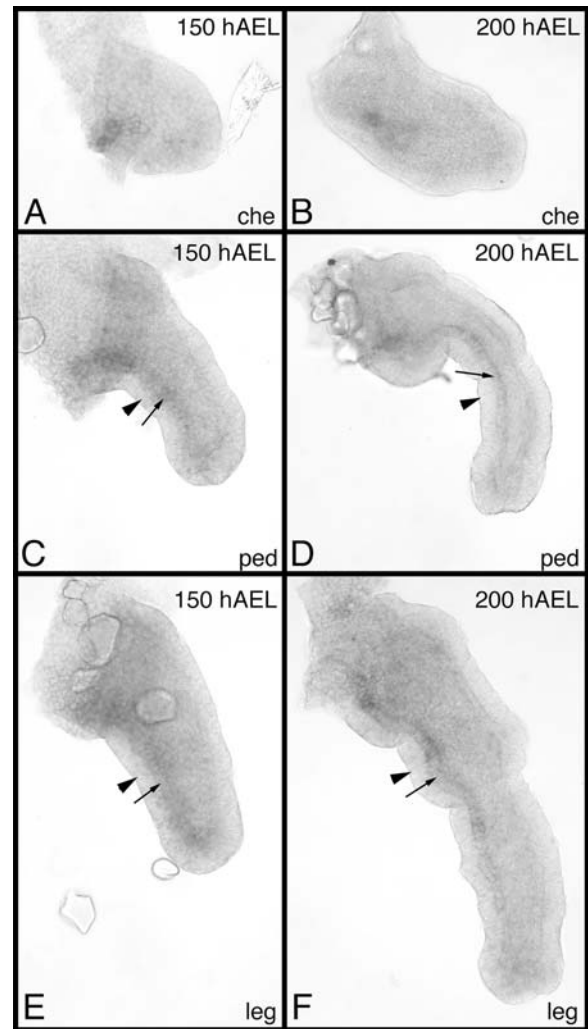


Fig. 3 Expression of *Cs-Notum* during appendage development. **A, B** Chelicera at approximately 150 and 200 h AEL, respectively. **C, D** Pedipalp at approximately 150 and 200 h AEL, respectively. **E, F** Walking leg at approximately 150 and 200 h AEL, respectively. Expression in the pedipalps and the legs is restricted to the ventral mesoderm (arrow in C–F), while the adjacent ectoderm does not express *Cs-Notum* (arrowhead in C–F). Expression in the chelicera is restricted to the proximal ventral mesoderm (A, B). All appendages are in anterior aspect and the proximal end is facing to the left. Abbreviations: che, chelicera; ped, pedipalp

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