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Gene expression pattern

Cloning and expression of *Drosophila SOCS36E* and its potential regulation by the JAK/STAT pathway

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Abstract

The suppressor of cytokine signalling (SOCS) gene family was originally identified as an immediate early response to cytokine signalling and function as negative regulators of the Janus kinase (JAK)/signal tranducers and activators of transcription (STAT) signal transduction pathway [Krebs and Hilton, J. Cell Sci. 113 (2000) 2813; Starr and Hilton, Int. J. Biochem. Cell Biol. 30 (1998) 1081]. Although key components of the *Drosophila* JAK/STAT pathway have been identified [Brown et al., Curr. Biol. 11 (2001) 1700, reviewed in Zeidler et al., Oncogene 19 (2000) 2598], regulators of the pathway, and SOCS genes in particular, have not yet been characterised. Here we report the cloning of *Drosophila SOCS36E* and show its expression pattern during embryonic and imaginal disc development. *SOCS36E* is expressed in an essentially identical pattern to the *Drosophila* JAK/STAT pathway ligand unpaired (Upd). It is not expressed in *upd* mutant embryos and is upregulated in response to ectopic activation of the pathway during both embryonic and imaginal development.

Keywords: *SOCS36E*; Suppressor of cytokine signalling; SOCS-5; CIS; Janus kinase; Signal tranducers and activators of transcription; STAT92E; Unpaired; Domeless; *Drosophila melanogaster*; marelle; hopscotch; SH2

1. Results

1.1. Cloning and genomic organisation of SOCS36E

Basic local alignment search tool (BLAST) searches of the *Drosophila* genome sequence (Adams et al., 2000) identified three putative suppressor of cytokine signalling (SOCS) genes which have been annotated and named according to their chromosomal position (see http://flybase.bio.indiana.edu/).

Two independent, putatively full length, expressed sequence tag (EST) clones of one homologue, *SOCS36E*, were sequenced to confirm the previously reported protein sequence (see accession number XM079441). This revealed the 5′ untranslated region of *SOCS36E* to be encoded by one exon previously ascribed to CG17681 (Fig. 1A). The *SOCS36E* region is flanked by other genes and includes 19 potential STAT92E consensus binding sites (red crosses in Fig. 1A; Yan et al., 1996). SOCS36E protein shares an overall identity of 29.7% to its closest vertebrate homologue, mouse SOCS-5 (Hilton et al., 1998) with a higher homology in the SH2 and SOCS domains (Fig. 1B). Similar levels of conservation are found between members of the vertebrate gene family.

1.2. Expression of SOCS36E

While in situ hybridisation using *SOCS36E* sense probes

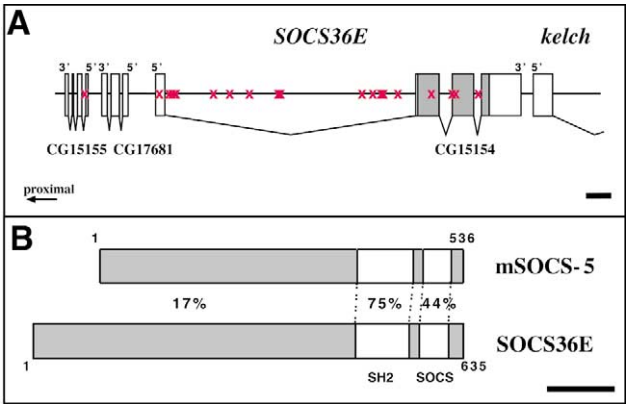

Fig. 1. (A) The genomic organisation of the *SOCS36E* region. Intron/exon structure for *SOCS36E* is experimentally derived, other genes are shown as predicted by the *Drosophila* genome project. The open reading frame of *SOCS36E* is indicated in grey. Scale bar represents 1 kb. (B) Domain scale alignment of mouse SOCS-5 and *Drosophila SOCS36E* showing domain structure and degree of sequence identity between different regions. Scale bar represents 100 amino acids.

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showed no signal (not shown), anti-sense probes revealed a dynamic and complex pattern strikingly similar to the JAK/STAT pathway ligand unpaired (*upd*). Given this similarity and the limited description of *upd* expression, published previously (Harrison et al., 1998), we show stage matched expression patterns of both *SOCS36E* and *upd* (Fig. 2). Expression of both the genes is first visible at stage 5 in a head stripe and a broad central domain (Fig. 2A), this pattern then resolves first into seven and then 14 stripes (Fig. 2B). Only *SOCS36E* is transiently upregulated in the presumptive mesoderm during gastrulation (Fig. 2C). Although both *upd* and *SOCS36E* are maintained in stripes until early stage 9 (Fig. 2D), *upd* stripes are one to two cells wide (insert Fig. 2D′) while *SOCS36E* expression is four to five cells wide (insert in Fig. 2D), a pattern consistent with JAK/STAT pathway activity presumed to result from diffusion of the extracellular Upd ligand.

Expression in a subset of neuroblasts is transiently observed during stage 9 (Fig. 2E) immediately after the stripes fade in medial regions to leave ventro-lateral expression in a ring centred around the tracheal pits (Fig. 2F). During stage 12, expression is maintained in the trachea

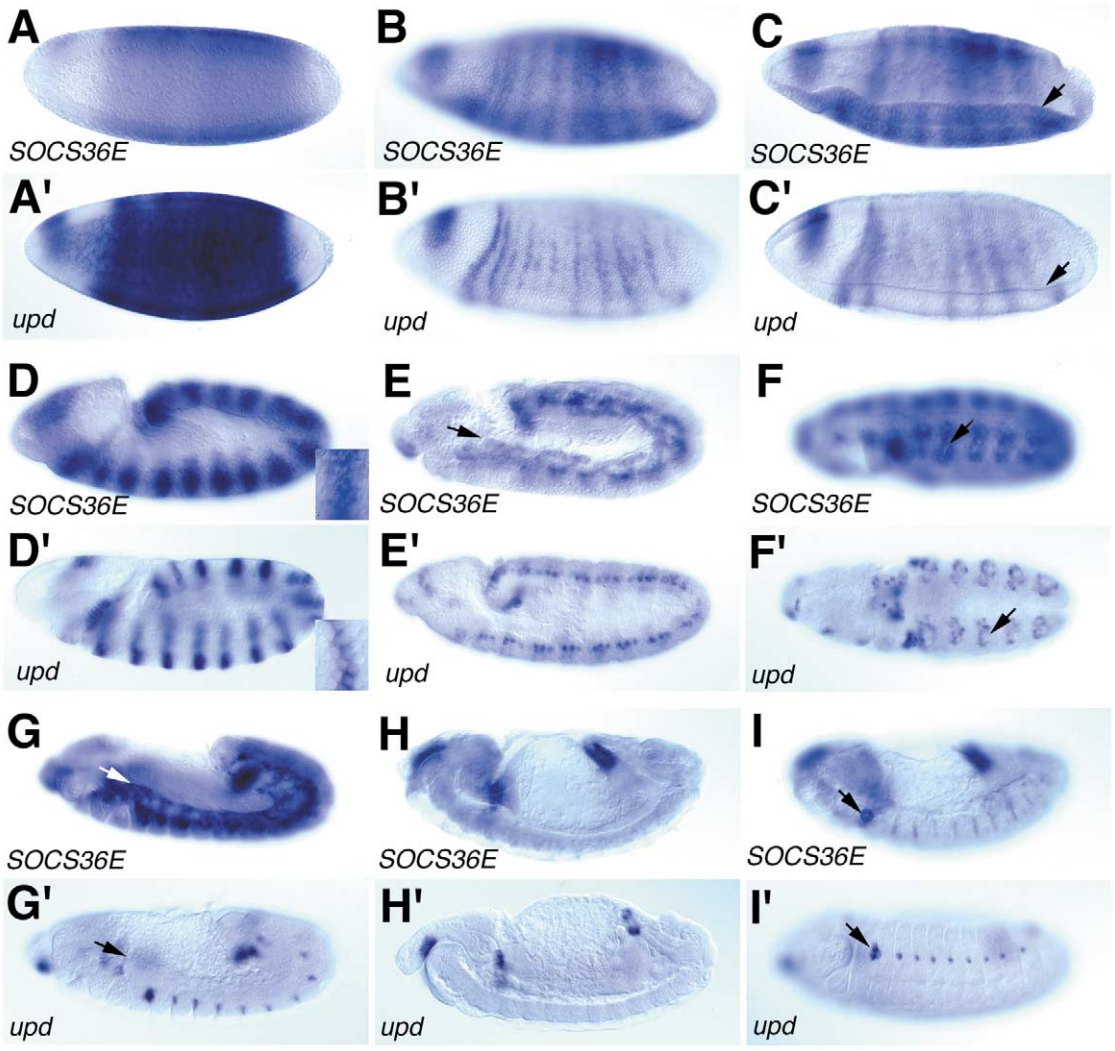

Fig. 2. The expression of *SOCS36E* (A–I) and *upd* (A′–I′) during embryogenesis are shown as stage matched pairs, with the probes used, marked in each panel. Unless noted otherwise, embryos are shown anterior to the left and dorsal up. (A) Stage 5 embryo showing staining in the dorsal anterior head region and throughout much of the trunk. (B, C) The same stage 6 embryos focused laterally (B) and internally (C) showing expression in the head region, in two stripes flanking the presumptive cephalic furrow, and seven diffuse stripes in *SOCS36E* and 14 narrow stripes through the trunk region in *upd*. Only *SOCS36E* expression is detected in the invaginating mesoderm (arrows in C). (D) Early stage 9 embryo showing staining anterior to the ventral cephalic furrow, in the head region and in 14 stripes. Inserts show higher magnification of single stripes. (E) Stage 10 expression is transiently detected in three neuroblasts per-hemisegment and *SOCS36E* begins to be expressed at the leading edge cells (arrow in E). (F) Dorsal/ventral views of late stage 10 embryos show a ring of expression surrounding the tracheal pits (arrows). (G) Stage 12 embryos show expression in the trachea, in the clypeolabrum and the hindgut (out of focus). *SOCS36E* expression in the leading edge cells (arrow in G) is strong and not detected using *upd* probes (arrow in G′). (H, I) Expression in stage 14 embryos is detected in the clypeolabrum, proventriculus and hindgut. Expression in the anterior spiracle (arrows) and tracheal pits is maintained with *upd* detected as dots on the lateral flanks (I′) and *SOCS36E* as stripes at more medial layers (I).

and *SOCS36E* (but not *upd*) is expressed in leading edge cells (Fig. 2G). During stages 14–15, expression is limited to the inner clypeolabrum, the proventriculus, the hindgut (Fig. 2H) as well as the anterior and medial spiracles (Fig. 2I).

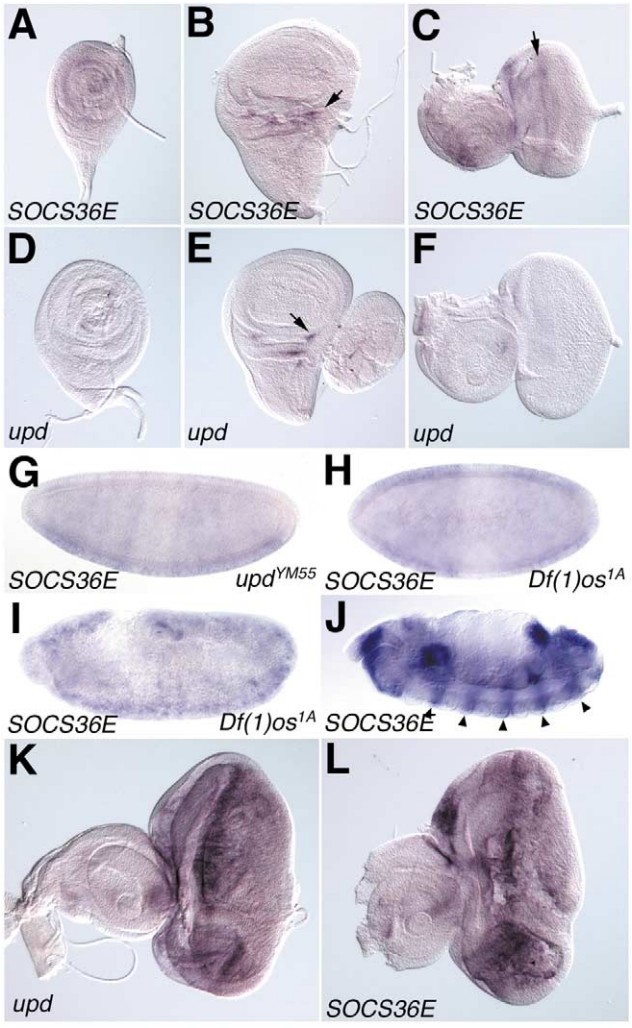

Fig. 3. *SOCS36E* and *upd* expression in late third instar imaginal discs and the control of *SOCS36E* expression. Discs are stained for expression of the indicated transcript. Embryos are shown anterior left and dorsal up. (A) Leg imaginal disc stained for *SOCS36E* expression. (B) Wing disc showing *SOCS36E* expression in regions of the dorsal hinge region (arrow). (C) Eye-antennal disc complex showing expression in the antennal disc and the morphogenetic furrow (arrow). (D) Leg imaginal disc showing weak *upd* staining in distal regions. (E) Wing disc showing *upd* expression in three regions within the dorsal hinge region (arrow). (F) Eye-antennal disc complex showing expression of *upd* at the posterior edge of the antennal disc. (G,H) Stage 5 embryos hemizygous mutant for upd^{YM55} (G) and the deficiency $Df(1)os^{1A}$ (H) show greatly reduced *SOCS36E* staining (compare to Fig. 2A). (I) A $Df(1)os^{1A}$ hemizygous embryo at stages 8/9 showing only weak speckled staining. The strong epidermal stripes seen in wild type (Fig. 2D) are not detected. (J) A stage 13 embryo carrying *paired-Gal4/UAS-*hop^{Tuml} and stained for *SOCS36E* expression, shows both the endogenous expression (see Fig. 2H) and stripes expressed in every alternate segment (arrowheads). (K,L) Late third instar eye antennal imaginal disc complexes carrying a *GMR-upd* transgene, are overgrown and show very strong expression of *upd* (E) and *SOCS36E* (F).

During late third instar imaginal disc development, *SOCS36E* expression is detected in the leg disc (Fig. 3A), regions of the dorsal wing hinge (Fig. 3B), in the antennal disc and in the morphogenetic furrow of the developing eye (Fig. 3C). With the exception of the wing hinge domains, the pattern of *upd* expression (Fig. 3D–F) is less similar to *SOCS36E* during imaginal development.

1.3. JAK/STAT signalling is necessary and sufficient for most SOCS36E expression

Given the similarity of *SOCS36E* and *upd* expressions, and the potential STAT92E binding sites within *SOCS36E*, we tested whether embryonic *SOCS36E* expression was *upd* dependent. In *upd* mutant embryos *SOCS36E* expression at stage 5 is only very weakly detectable in embryos hemizygous for the hypomorphic, nonsense mutation upd^{YM55} (Fig. 3G) or $Df(1)os^{1A}$ (Fig. 3H) a small deficiency removing the genomic region (Harrison et al., 1998; Eberl et al., 1992). With the exception of weak punctate expression not detected in wild type, *SOCS36E* is entirely missing at stage 9 in $Df(1)os^{1A}$ backgrounds (Fig. 3I, compare with Fig. 2D).

SOCS36E expression can also be upregulated by ectopic JAK/STAT pathway activation. Using the Gal4/UAS system (Brand and Perrimon, 1993) and a *paired-Gal4* driver line (gift of N. Perrimon) embryos, expressing the gain of function JAK allele hop^{Tuml} (Harrison et al., 1995) in alternate stripes were found to ectopically express *SOCS36E* in a pair-rule like pattern (arrow heads in Fig. 3J). In addition eye imaginal discs expressing *upd*, under the control of the GMR promoter (Fig. 3K; Ellis et al., 1993) showed greatly increased levels of *SOCS36E* expression (Fig. 3L).

Taken together, it seems that JAK/STAT pathway activity is responsible for much of the *SOCS36E* expression during *Drosophila* development and is sufficient to cause ectopic expression. However, *SOCS36E* expression, not apparently associated with JAK/STAT pathway activity, does exist and may reflect either other transcriptional control mechanisms or JAK/STAT pathway activation by as yet uncharacterised ligands or inter-pathway crosstalk.

2. Materials and methods

upd mutant lines described by Harrison et al. (1998) were balanced using chromosomes expressing β-galactosidase under control of the *ftz* promoter (see http://flystocks.bio.indiana.edu/ for details) to identify hemizygous mutant male embryos. Glass multimerised response (GMR)–*upd* transformants were a kind gift of Erika Bach and will be described elsewhere. Developmental stages are described in Campos-Ortega and Hartenstein (1997).

EST clones LD22121 and SD04320, partially sequenced by the Berkley *Drosophila* Genome Project (www.fruitfly.org), were obtained from Research Genetics (Huntsville,

AL) and sequenced on both strands. Sequences were assembled and analysed using DNA Star software together with alignments performed at http://www.ebi.ac.uk/emboss/align/.

RNA probes were synthesised from linearised EST clones using DIG RNA labelling kit (Roche). In situ staining was performed as described by Lehmann and Tautz (1994).

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