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Gene expression pattern

# Localized expression of the *Drosophila* gene *Dxl6*, a novel member of the serine/arginine rich (SR) family of splicing factors

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**Abstract**

Members of the highly conserved family of serine/arginine rich (SR) splicing factors play an essential role in the recognition of the exonic splicing enhancers that control the choice of splice sites in primary transcripts. Here, we report the cloning and the expression pattern of *Dxl6*, a novel *Drosophila* member of this protein family. *Dxl6* is located on the second chromosome in a position next to *hrp48* and *Dwee1*. Its intron contains Dnop5, a small nucleolar ribonucleoprotein (snoRNP) which is essential for rRNA-processing. During oogenesis, *Dxl6* transcripts are expressed in nurse cells. Transcripts are transported into the oocyte and maintained in a ubiquitous pattern in the egg and early embryo. Zygotic *Dxl6* transcripts accumulate in the neuroectodermal region of the gastrulating embryo and become highly enriched in the central nervous system (CNS) and brain of embryos. During larval stages, *Dxl6* transcripts are detected in distinct patterns in the developing imaginal discs. 

*Keywords:* Splicing; Serine/arginine rich; SR factor; Neuroectoderm expression; Central nervous system

## 1. Results

Splicing of nuclear pre-mRNA depends on the formation of RNA/protein complexes, called spliceosomes. It involves the recognition of intron sequences by multiple transacting factors (Krämer, 1996; Staley and Guthrie, 1998). Recognition sequences within the exon downstream of the intron are recognized by serine/arginine-rich proteins (SR) which are highly conserved in metazoa. SR proteins not only play an essential role in general splicing, but also in the selection of alternative splice sites (Manley and Tacke, 1996). They exert a bipartite structure consisting of one or two N-terminal RNA binding domains necessary for the sequence-specific binding to splicing enhancer sequences and C-terminal serine/arginine repeats which participate in the localization of the proteins to subnuclear structures (Graveley et al., 1999; Tacke and Manley, 1999).

During analysis of a genomic DNA interval at position 27C4-5 on the left arm of second chromosome of *Drosophila*, we identified a transcription unit which codes for a novel *Drosophila* SR homologue, referred to as *Dxl6* (Fig. 1A). *Dxl6* is located between the *hrp48* and the *Drosophila wee1* kinase genes. Sequence analysis of two *Dxl6* cDNAs (LD02483 and GM07743; obtained from Berkley *Drosophila* Genome Project/HHMI EST Project, unpublished) and the corresponding genomic DNA revealed that the transcription unit codes for two exons. The exons are separated by a 4163 bp intron which contains the *Dnop5* transcription unit coding for a member of the nop5/sik1 protein family (Fig. 1A) which has an essential function in rRNA-processing (Vorbrüggen et al., 1999). The two characterized cDNAs code for transcripts which differ by 90 bp in their 3′ untranslated region due to different polyadenylation signals. Their single open reading frames translate into a 258 amino acids polypeptide similar to the SR splicing factors with two N-terminal RNA-binding domains (Fig. 1A,B). Phylogenetic analysis (Fig. 1D) indicates that the newly identified *Drosophila* member of SR proteins is the closest relative of mouse Xl6 (therefore the name) and human SFRS3 (Ayane et al., 1991; Zahler et al., 1992).

Fig. 2 summarizes the *Dxl6* expression pattern as revealed by in situ hybridization with cDNA probes to whole mount preparations of ovaries, embryos and imaginal discs of the larvae. *Dxl6* transcripts are detected in nurse cells during all stages of oogenesis (Fig 2A). They become increasingly enriched in the oocytes, indicating their trans-

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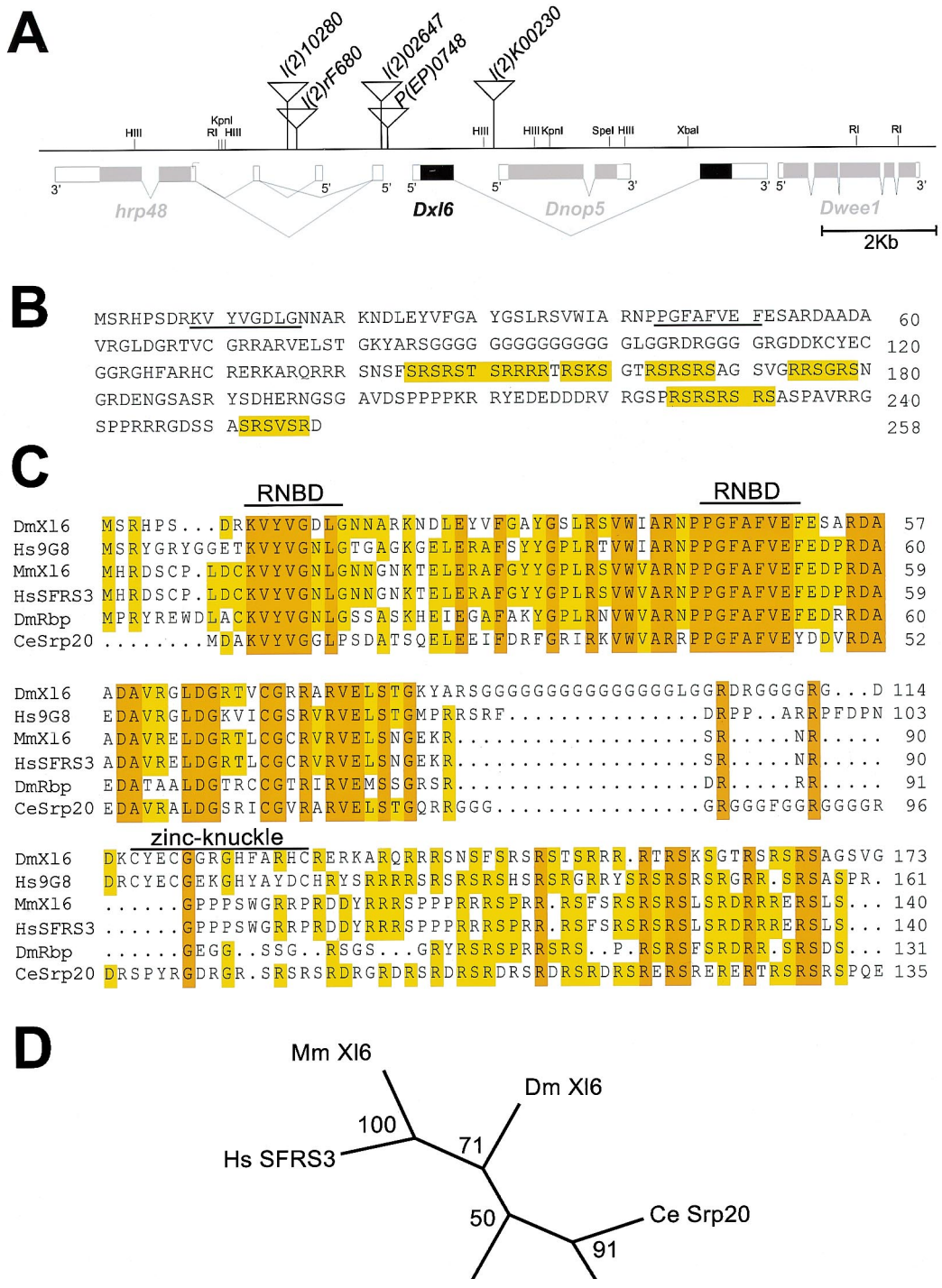


Fig. 1. Physical map of the *Dxl6* transcription unit and protein sequence. (A) Genomic organization of *Dxl6*. Diagnostic restriction sites are indicated; *Eco*RI (RI), *Hin*dIII (HIII), *Kpn*I, *Xba*I and *Spe*I. Sites of P element insertions are marked by triangles. Exon-intron boundaries were determined by sequence comparison (cDNA versus genomic sequence of the P1 DS01321; accession no. AC004277). (B) Protein sequence of DXl6. Potential RNA binding domains are underlined, the SR domains are marked in yellow (accession no. AJ249466). (C) Amino acid sequence alignment of DXl6, human SFSR3 (Zahler et al., 1992), mouse Xl6 (Ayane et al., 1991), human 9G8 (Cavaloc et al., 1994), *Drosophila* Rbp1 (Kim et al., 1992) and *C. elegans* Srp20 (accession no. U41007) as obtained by ClustalW program (Thompson et al., 1994). RNBD refers to two RNA binding domains. Identical amino acid residues are marked in orange, conserved residues in yellow. (D) Evolutionary relationship of SR proteins as revealed by neighbour joining analysis. Numbers refer to Bootstrap percentages obtained from neighbour-joining.

port from the nurse cells. Transcripts remain present at high levels in eggs and early embryos up to the gastrulation stage (Fig. 2B,C). Discernible zygotic expression is first detected during gastrulation, covering the area of the differentiating

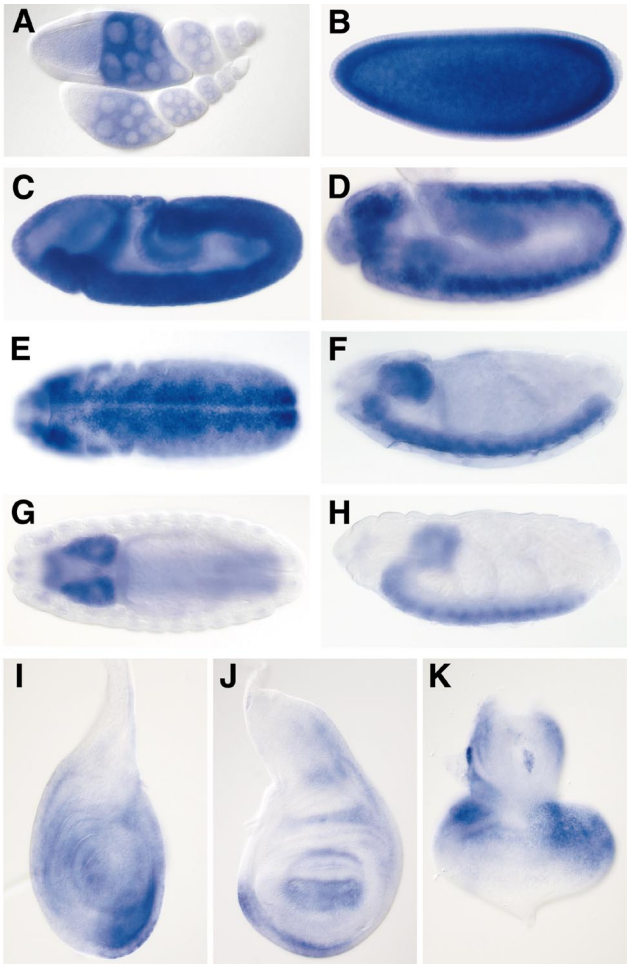


Fig. 2. Transcript pattern of the *Dxl6* gene visualized by in situ hybridization of *Dx16* cDNA to whole mount preparations ovaries (A), to embryos at embryonic stages 5 (B), 9 (C), 11 (D, E), 14 (F,G) and 16 (H) as well as leg (I), wing (J) and eye-antenna (K) imaginal discs of third instar larvae. Wildtype embryos are shown in lateral (B–D,F,H) or dorsal view (E,G). Orientation of embryos is anterior to the left. Details on the expression patterns are provided in the text. Stages of embryos according to (Campos-Ortega and Hartenstein, 1985).

neuroectoderm of stage 11 embryos (Fig. 2D,E). At late stage 14, *Dxl6* transcripts are highly enriched if not exclusively expressed in the central nervous system (CNS) and brain of the embryo (Fig. 2F,G) where they remain prominently throughout the later stages of embryogenesis (Fig. 2H).

In developing imaginal discs of the larvae, weak and ubiquitous *Dx16* expression can be detected. In addition, transcripts become enriched in distinct spatial patterns showing high amount of transcripts in the dorsal sector of the leg disc (Fig. 2I), in the ventral pouch as well as the hinge and pleura regions of the wing disc (Fig. 2J) and in ventral and dorsal locations of the eye and antenna portions of the combined eye-antenna disc (Fig. 2K). In conclusion, zygotic *Dx16* expression in the embryo is neurospecific whereas in the larvae, transcripts are also found in areas of the imaginal discs that differentiate epidermis.

## 2. Materials and methods

Cloning of the *Dxl6* gene was initiated by the characterization of the genomic plasmid rescue fragment of the *l(2)10280* P element (Karpen and Spradling, 1992). In situ hybridization analysis of whole mount embryos with different subfragments enabled us to identify two genes, *Dxl6* and *Dnop5*. Sequence comparison of two cDNAs encoding *Dxl6*, LD02483 and GM07743, (Berkley *Drosophila* Genome Project/HHMI EST Project, unpublished) with the genomic sequence of this locus helped to characterize the exon/intron structure of the *Dxl6* gene locus. Both cDNAs were used for the preparation of antisense digoxygenin-labelled RNA probes according to the protocol of the manufacturer (Boehringer). In situ hybridizations were performed as described (Klingler and Gergen, 1993). The map in Fig. 1A is based on the sequence of the genomic P1 clone DS01321 (accession no. AC004277) (Kimmerly et al., 1996). Molecular phylogenetic trees were constructed from protein sequences using the PHYLIP 3.572c software (Phylogenetic Interference Package) and robustness was assessed by 500 bootstrap resamplings.

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