ELSEVIER

Mechanisms of Development 90 (2000) 305–308

www.elsevier.com/locate/modo

Gene expression pattern

Restricted expression and subnuclear localization of the *Drosophila* gene *Dnop5*, a member of the Nop/Sik family of the conserved rRNA processing factors

Gerd Vorbrüggen[1,*], Susanne Önel[1,2], Herbert Jäckle

Max Planck Institut für biophysikalische Chemie, Abteilung Molekulare Entwicklungsbiologie, Am Fassberg 11, D-37077 Göttingen, Germany

Received 15 September 1999; accepted 23 September 1999

Abstract

Members of the conserved *nop5/sik1* gene family encode components of small nucleolar ribonucleoprotein (snoRNP) complexes, which have an essential function in rRNA-processing. We describe a novel *Drosophila* member of this family, termed *Dnop5*. The gene is expressed in nurse cells during oogenesis and transcripts are deposited into the growing oocyte. Maternal transcripts become evenly distributed in the egg and remain in a ubiquitous pattern during early embryogenesis. Zygotic *Dnop5* expression is initiated during the extended germband stage. Transcripts accumulate in mesoderm and midgut primordia, and in the developing imaginal discs of the larvae. Consistent with a function in rRNA processing, *Dnop5* protein (DNop5) accumulates in a nuclear substructure, likely to be the nucleolus. Maternal protein accumulates in the nucleolus of all cells in the early embryo, whereas DNop5 that is derived from zygotic mRNA, is restricted to the nuclei of muscles and midgut. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: rRNA processing; Muscle; Mesoderm; Nop; Sik; Nucleolus; Midgut

Results

In an attempt to isolate genes essential for *Drosophila* mesoderm differentiation, we screened P element enhancer trap insertions looking for β-galactosidase expression patterns in the mesoderm and its derivatives. Embryos derived from the P line *l(2)10280* (Karpen and Spradling, 1992) showed strong β-galactosidase expression in somatic muscles. Cloning of the insertion site by plasmid rescue (Wilson et al., 1989) revealed that the P element resides at position 27C4-5 on the left arm of the second chromosome. The region contains four transcription units (Fig. 1A) including the *hrp48* and the *Drosophila wee1* gene (Matunis et al., 1992; Campbell et al., 1995). The P element of line *l(2)K00230* (Torok et al., 1993), which shows a *l(2)10280*-like β-galactosidase expression pattern, was inserted about 100bp 5′ of the transcription unit *Dnop5* (Fig. 1A). The β-galactosidase expression of line *l(2)K00230* (Fig. 2A) and the coinciding patterns of *Dnop5* transcripts (see below) indicate that the P element has trapped the *Dnop5* enhancer.

We isolated an almost full size *Dnop5* cDNA clone which codes for a transcript of approximately 1800 nucleotides (Fig. 1D). Conceptual translation of its open reading frame predicts a polypeptide of 510 amino acids (Fig. 1B). Sequence comparison with known proteins indicates that Dnop5 protein is a member of the Nop5/Sik protein family. Nop5/Sik proteins are components of small ribonucleoprotein complexes (snoRNPs) and play a role in rRNA-processing (Maxwell and Fournier, 1995). As other family members, DNop5 contains multiple KKX motifs at the carboxy terminus, a motif also found in microtuble-binding proteins (reviewed in Maccioni and Cambiazo, 1995). Functional studies on yeast *nop5/sik* genes have shown that mutations interfere with the processing of the 18s rRNA and cause reduced cell growth and proliferation (Gautier et al., 1997; Wu et al., 1998). Phylogenetic analysis shows that the Nop5/Sik1 family members are highly conserved from plants to human and that DNop5 is the closest relative of Nop5 of *Caenorhabditis elegans* (Fig. 1C,E).

We examined the spatiotemporal patterns of *Dnop5* transcript and protein expression by probing ovaries, embryos and larval imaginal discs with *Dnop5* cDNA and anti-DNop5 antibodies, respectively. During oogenesis, transcripts appear in both nurse cells and follicle cells (Fig. 2B). They subsequently accumulate in the oocyte, implying

* Corresponding author.

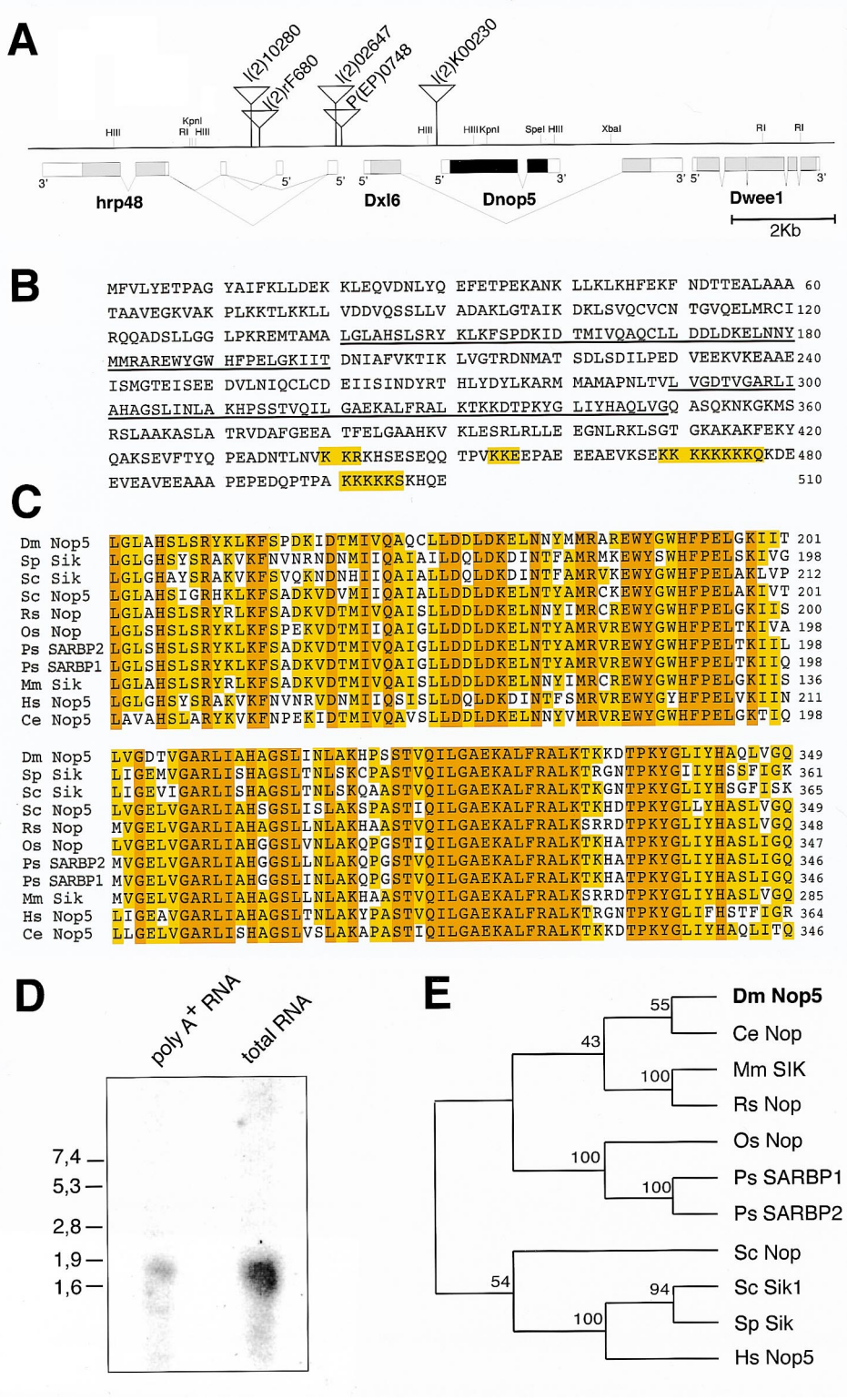
[1] Both authors contributed equally.

[2] Present address: Universität Münster, Institut für Neurobiologie, Badestrasse 9, D-48149 Münster, Germany.

0925-4773/00/$ - see front matter © 2000 Elsevier Science Ireland Ltd. All rights reserved.
PII: S0925-4773(99)00249-X

transport from nurse cells to oocyte. After egg deposition, maternal *Dnop5* transcripts were ubiquitously distributed in eggs and in embryos during syncytial development (not shown). From blastoderm stage onwards, the maternally derived transcripts decrease strongly and escape detection by the end of gastrulation.

Zygotic *Dnop5* expression was first detected in the mesoderm anlage during early gastrulation (Fig. 2C). Subsequently, zygotic transcripts appear in mesoderm (Fig. 2D) and the invaginating midgut primordia (Fig. 2E). During germband retraction, *Dnop5* transcripts accumulate in the developing midgut where they remain in high amounts until the hatching of the larva (Fig. 2F,H). Transcripts become less prominent in mesoderm derivatives from germband



retraction stage onwards (Fig 2G,H; data not shown). During the larval stage, *Dnop5* transcripts are found in the developing imaginal discs. In leg imaginal discs, low amounts of transcripts are found in a ubiquitous pattern (not shown). In the wing disc, the transcripts are highly enriched in the posterior compartment (Fig. 2I), whereas ubiquitous low amounts of transcripts were observed in eye-antenna discs including areas of high expression. (Fig. 2K). Transcripts were also prominent in distinct areas of the larval brain (Fig. 2J). Thus, whereas zygotic transcription of *Dnop5* in embryos is restricted to mesodermal and endodermal germlayer derivatives, larval *Dnop5* expression occurs in restricted patterns in brain and imaginal discs which are of ectodermal origin.

DNop5, which derives from maternal RNA, accumulates in the nuclei of syncytial blastoderm embryos (Fig. 2L). It appears in a subnuclear structure likely to be the nucleolus. (Fig. 2M,N). The nucleolar pattern of the maternal DNop5 complement is maintained in ectodermal cells until the onset of germband retraction. At late stages of embryogenesis, DNop5 is found exclusively in nuclei of somatic muscles and midgut cells (Fig. 2O), corresponding to the sites of zygotic *Dnop5* expression.

Materials and methods

Cloning of the *Dnop5* gene was initiated by plasmid rescue (Wilson et al., 1989) using the *l(2)10280* P element. The resulting 5.5 kbp genomic fragment was used to screen an embryonic cDNA library (Stratagene). The longest cDNA isolate (1790 bp) was sequenced and used to prepare digoxygenin-labelled antisense RNA probes as outlined by the manufacturer (Boehringer, Manheim). In situ hybridization of wholemount embryos, ovaries and imaginal discs of third instar larvae were performed as described (Klingler and Gergen, 1993). His-tagged DNop5 full-length fusion protein was expressed in BL-21(DE3) bacteria, purified under denaturing conditions using nickel affinity chromatography according to the manufacturer's protocol (Invitrogene) and used to generate rabbit antibodies (Eurogentec, Brussels). Antibody staining was performed as described (Macdonald et al., 1986).

The locus has been sequenced as part of the genomic P1 clone (accession no. AC004277). The alternative spliced transcripts of the *hrp48* (Matunis et al., 1992) in Fig. 1A

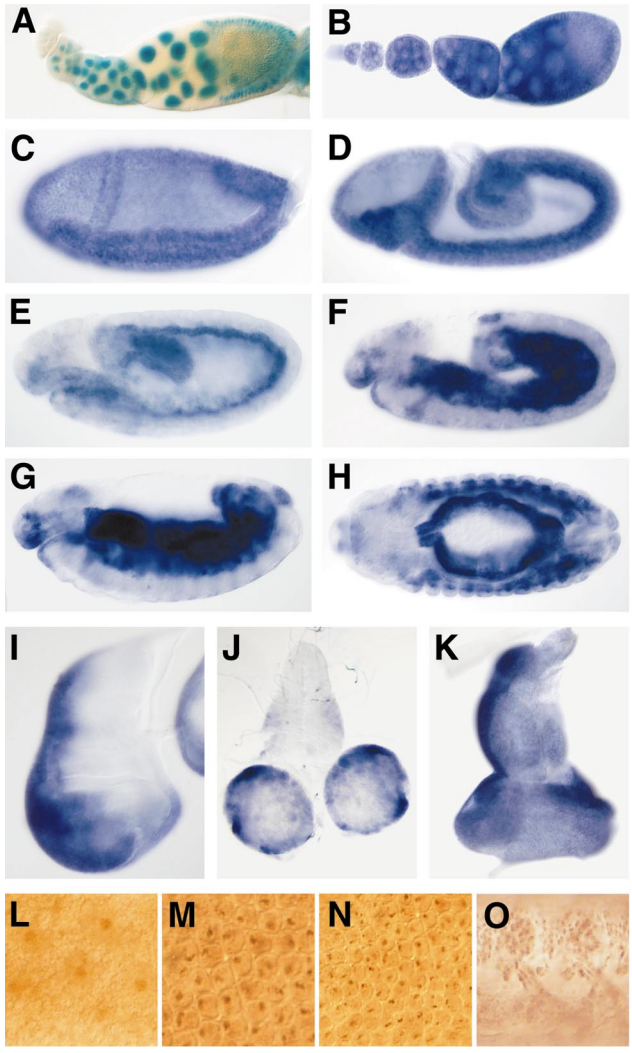


Fig. 2. Expression pattern of *Dnop5* transcripts and protein. (A) β-galactosidase activity staining in ovaries of flies carrying the *l(2)K00230* P element. (B–K) *Dnop5* transcript pattern as revealed by in situ hybridization using digoxygenin labelled RNA antisense probes. Shown are ovaries (B), lateral views of embryos (anterior to the left and dorsal up; staging according to (Campos-Ortega and Hartenstein, 1985) at stage 6 (C), stage 8 (D), stage 11 (E), stage 12 (F), lateral and dorsal views of embryos at stage 13 (G and H) as well as a wing disc (I), brain (J) and eye-antenna disc (K) of third instar larvae. (L–O) Enlargements of wholemounts stainings showing DNop5 protein expression in nuclei of embryos at early syncytial blastoderm stage (L), cellular blastoderm (M), gastrulation (N) and in the nuclei of somatic muscles of embryos at stage 16 (O). Note protein staining in a subnuclear structure likely to be the nucleolus. For details see text.

Fig. 1. Genomic organization *Dnop5* and protein sequence. (A) Genomic organization of *Dnop5*. The map shows the restriction sites *Eco*RI (RI), *Hin*dIII (HIII), *Kpn*I, *Xba*I and *Spe*I. P element insertions are indicated by triangles; the plasmid rescue fragment of the *l(2)10280* P element contained genomic sequences up to the SpeI site. Exon/intron boundaries were established by comparing cDNA and genomic sequence (P1 accession no. AC004277). (B) DNop5 protein sequence (accession no. AJ249465); aligned portion (C) are underlined. Yellow boxes: KKX motifs characteristic for microtuble binding proteins. (C) Alignment of partial sequences of Nop5 family members. Sp Sik (accession no. AL035216), Sc Sik (Morin et al., 1995), Sc Nop5 (Wu et al., 1998), Rs Nop (accession no. AF069782), Os Nop5 (accession no. AB015431), Ps SARBP1 (accession no. AF061962) Ps SARBP2 (accession no. AF061963), Mm Sik (accession no. 2996194), Hs Nop (accession no. Y12065) and Ce Nop5 (accession no. AF043704) as obtained by ClustalW program (Thompson et al., 1994). Identical and conserved amino acid residues are marked in orange and yellow, respectively. (D) Northern Blot of *Dnop5* RNA-expression using poly(A)$^+$ RNA and total RNA from 0–18 h old embryos. (E) Evolutionary relationship of Nop5/Sik proteins, as obtained by neighbour joining analysis. Numbers refer to Bootstrap percentages obtained from neighbour joining.

are based on three cDNA clots (Berkley *Drosophila* Genome Project/HHMI EST Project, unpublished). Molecular phylogenetic trees were constructed from protein sequences using the PHYLIP 3.572c software (Phylogenetic Interference Package) and the robustness was assessed by 500 bootstrap resamplings.

Acknowledgements

We thank our colleagues in the laboratory for their various contributions and G. Dowe for sequencing. This work has been supported by the Deutsche Forschungsgemeinschaft (SFB 271).

References

Campbell, S.D., Sprenger, F., Edgar, B.A., O'Farrell, P.H., 1995. *Drosophila* Wee1 kinase rescues fission yeast from mitotic catastrophe and phosphorylates *Drosophila* Cdc2 in vitro. Mol. Biol. Cell 6, 1333–1347.

Campos-Ortega, J.A., Hartenstein, V., 1985. The Embryonic Development of *Drosophila melanogaster*. Springer, Berlin, 227 pp.

Gautier, T., Berges, T., Tollervey, D., Hurt, E., 1997. Nucleolar KKE/D repeat proteins Nop56p and Nop58p interact with Nop1p and are required for ribosome biogenesis. Mol. Cell. Biol. 17, 7088–7098.

Karpen, G.H., Spradling, A.C., 1992. Analysis of subtelomeric heterochromatin in the *Drosophila* minichromosome Dp1187 by single P element insertional mutagenesis. Genetics 132, 737–753.

Klingler, M., Gergen, J.P., 1993. Regulation of runt transcription by *Drosophila* segmentation genes. Mech. Dev. 43, 3–19.

Maccioni, R.B., Cambiazo, V., 1995. Role of microtubule-associated proteins in the control of microtubule assembly. Physiol. Rev. 75, 835–864.

Macdonald, P.M., Ingham, P., Struhl, G., 1986. Isolation, structure, and expression of even-skipped: a second pair- rule gene of *Drosophila* containing a homeobox. Cell 47, 721–734.

Matunis, E.L., Matunis, M.J., Dreyfuss, G., 1992. Characterization of the major hnRNP proteins from *Drosophila melanogaster*. J. Cell Biol. 116, 257–269.

Maxwell, E.S., Fournier, M.J., 1995. The small nucleolar RNAs. Annu. Rev. Biochem. 64, 897–934.

Morin, P.J., Downs, J.A., Snodgrass, A.M., Gilmore, T.D., 1995. Genetic analysis of growth inhibition by GAL4-L kappa B-alpha in *Saccharomyces cerevisiae*. Cell Growth Diff. 6, 789–798.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Torok, T., Tick, G., Alvarado, M., Kiss, I., 1993. P-lacW insertional mutagenesis on the second chromosome of *Drosophila melanogaster*: isolation of lethals with different overgrowth phenotypes. Genetics 135, 71–80.

Wilson, C., Pearson, R.K., Bellen, H.J., O'Kane, C.J., Grossniklaus, U., Gehring, W.J., 1989. P-element-mediated enhancer detection: an efficient method for isolating and characterizing developmentally regulated genes in *Drosophila*. Genes Dev 3, 1301–1313.

Wu, P., Brockenbrough, J.S., Metcalfe, A.C., Chen, S., Aris, J.P., 1998. Nop5p is a small nucleolar ribonucleoprotein component required for pre- 18 S rRNA processing in yeast. J. Biol. Chem. 273, 16453–16463.