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Alternative transcripts expressed by *small bristles*, the *Drosophila melanogaster nxf1* gene

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ABSTRACT

The tissue-specific accumulation of *small bristles (Dm nxf1)* transcripts at different developmental stages of *Drosophila melanogaster* was analyzed by Northern blots and RT PCR. We identified four distinct transcripts: ubiquitous (3.5 kb); ovary and early embryo specific (3.3 kb); testis specific (1.9 kb and 2.8 kb) and nervous system specific (5.1 kb). The pattern of *Dm nxf1* gene expression in ovaries and early embryos (0–2 h) is similar: the sizes of transcripts range from 3.0 to 3.5 kb. We propose that this size variability may reflect the different extent of cytoplasmic polyadenylation. In testes, the 2.8-kb transcript originates from alternative termination of transcription and the 1.9-kb transcript is supposed to originate from an alternative transcription start. During ontogenesis, the 5.1-kb transcript can be clearly detected in 10- to 18-h-old embryos, most prominently in the nervous ganglia of larvae, and it represents a major species in imago head extracts. We found that the 5.1-kb transcript, similarly to the *nxf1* heavy transcripts in *Homo sapiens* and *Mus musculus*, results from the retention of intron 5–6 that corresponds to the intron 10–11 in *Hs nxf1* and *Mm nxf1* genes.

1. Introduction

The *small bristles (sbr)* gene of *D. melanogaster* belongs to an evolutionarily conserved family of genes known as *nxf* (nuclear export factor) (Herold et al., 2001; Tretyakova et al., 2001; Wilkie et al., 2001). The *sbr (Dm nxf1)* orthologs were found in various eukaryotic organisms: *Saccharomyces cerevisiae (Mex67)* (Segref et al., 1997), *Caenorhabditis elegans (Ce nxf1)* (Tan et al., 2000), *Mus musculus* (*Mm nxf1*) (Sasaki et al., 2005) and *Homo sapiens (tap/Hs nxf1)* (Kang and Cullen, 1999). The key function of *nxf1* genes is the export of general mRNAs from the nucleus to the cytoplasm (Herold et al., 2003), but for other members of the *nxf* family a role in further cytoplasmic fate of mRNAs has been proposed (Jun et al., 2001; Sasaki et al., 2005; Tretyakova et al., 2005).

In the yeast *S. cerevisiae* there is one *nxf* gene (*Mex67*), whereas the human genome contains six *nxf* family genes, while one of these (*Ψnxf6)* is a pseudogene (Herold et al., 2000). There are four *nxf* genes in *M. musculus* (Sasaki et al., 2005; Tretyakova et al., 2005; Tan et al., 2005). Since some of these paralogous genes are expressed in tissue-specific manner, the multiplication of *nxf* genes in metazoans may entail their functional specialization as the transport receptors of tissue-specific mRNAs (Herold et al., 2001; Yang et al., 2001). The mouse *nxf2* and *nxf3* are mostly expressed in testes, while *nxf3* and *nxf7* expression was also observed in brain cells (Wang et al., 2001; Yang et al., 2001; Sasaki et al., 2005). The human *nxf2* and *nxf3* are predominantly expressed in testes, and *nxf5* in fetal brain (Jun et al., 2001; Yang et al., 2001). Alternative splicing further contributes to the diversity of the *nxf* gene products (Herold et al., 2000; Yang et al., 2001; Sasaki et al., 2005).

D. melanogaster genome has four *nxf* family genes (Herold et al., 2000). In contrast to human *nxfs*, of which at least two (*nxf1* and *nxf2)* exhibit mRNA export activity, only *sbr* (*Dm nxf1*) mapped on the X-chromosome appears to be an active export receptor in *D. melanogaster* (Herold et al., 2001). The roles of the other three paralogs, *Dm nxf2-4*, are not yet established. Although Dm NXF2 has the typical NXF family structural domains as will be described below, this factor is not essential for general mRNA export in cell culture (Herold et al., 2001).

Mutations of *sbr* gene exhibit a variety of pleiotropic effects. Many of them can be explained in terms of alterations in mRNA export

Abbreviations: CPE, cytoplasmic polyadenylation element; CTE, constitutive transport element; LRR, leucine-rich repeat; NES, nuclear export signal; NLS, nuclear localization signal; NMD, nonsense-mediated mRNA decay; NPC, nuclear pore complex; NTF, nuclear transport factor; *nxf*, nuclear export factor; *sbr*, *small bristles*; UBA, ubiquitin-associated domain.

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function (Korey et al., 2001; Tretyakova et al., 2001; Wilkie et al., 2001). However the existence of allele-specific phenotypes may suggest that *sbr* gene has additional functions. Some of mutant alleles affect the morphology of bristles (Lindsley and Zimm, 1992; Zhimulev et al., 1981, 1982; Korey et al., 2001), while others lead to female sterility (Geer et al., 1983; Korey et al., 2001; Golubkova et al., 2004) or male sterility (Dybas et al., 1983; Geer et al., 1983), or lead to the increased frequency of chromosome non-disjunction (Mamon et al., 1990; Nikitina et al., 2003a) and long-term memory disorders (Nikitina et al., 2003b). Importantly, some of these allele-specific effects are dominant.

Such pleiotropy may be due in part to the production of different transcripts and/or proteins from the same gene, leading to additional functions. In this study, we show that *sbr* gene generates multiple transcripts. The transcriptional polymorphism, i.e. the existence of several transcripts corresponding to one gene, may underlie the different manifestations of *sbr* mutant alleles and lead to complex interallele interactions. The known sources of transcriptional polymorphism include alternative splicing (e.g., -intron retention), the use of alternative promoters and alternative polyadenylation sites. Some of the above have been reported for *nxf* gene family (Herold et al., 2000; Jun et al., 2001; Sasaki et al., 2005).

Here we show that *sbr* gene is capable of expressing several transcripts: 1.9 kb, 2.8 kb, 3.3 kb, 3.5 kb and 5.1 kb, while the only *sbr* mRNA reported previously is 3283 bases (GenBank AJ251947) (Korey et al., 2001; Wilkie et al., 2001). We propose that the 3.5-kb transcript is likely a polyadenylation variant of the 3.3-kb transcript. The 3.5-kb transcript appears to be expressed ubiquitously, with the exception of testes where its levels were undetectable on Northern blots, whereas the use of alternative initiation/termination of transcription gives rise to two testes-specific species (2.8 kb and 1.9 kb). The 2.8-kb transcript can potentially encode a full length SBR (672 a.a., 74 kDa). The 1.9-kb transcript originates from alternative transcription start in intron 3–4 and it might encode a novel isoform (536 aa, ~60 kDa), which may play a specialized role in spermatogenesis (Golubkova et al., unpublished). The 5.1-kb transcript that is mostly expressed in the nervous system includes the retained intron 5–6, similarly to the heavy transcripts of *Hs nxf1* and *Mm nxf1* that retain their corresponding intron 10–11.

Our work identified novel *sbr* transcripts that potentially encode testes as well as nervous system-specific SBR isoforms. We consider it possible that such SBR variants might play roles that are distinct from general mRNA export, consistent with the existence of allele-specific, pleiotropic phenotypes affecting the reproductive and nervous systems.

2. Materials and methods

2.1. D. melanogaster strain stock

The wild-type Oregon-R strain of *D. melanogaster* was analyzed. The flies were raised on standard yeast-raisin-agar medium at 25 °C. The saline solution for insect (Ephrussi and Beadle, 1936) was used for the dissection of *Drosophila* organs.

2.2. Isolation of RNA

Total RNA was isolated from the embryos, as well as the salivary glands and the nervous ganglia of third instar larva, and from the testes, ovaries and other body parts of the adult flies using the guanidine thiocyanate and phenol/chloroform extraction in a single step (Chomczynski and Sacchi, 1987).

2.3. Northern blot hybridization

The RNA samples (4–6 μg) were separated by 1.5–2% formaldehyde-agarose gel electrophoresis, transferred for 1.5 h onto Hybond-N + nylon membranes (Amersham) by VacuGene XL Vacuum blotter (Amersham) at 70 mbar, and UV cross-linked at 312 nm before prehybridization. The 6385-bp *Sac*I–*Xba*I genomic fragment (Fig. 1) of 14.5BB subclone from P1 clone DS03615 (as an insert into pBlue-ScriptSK$^-$), and *sbr* cDNA (GenBank accession no. AJ318090.I, cDNA fragment corresponding to SBR 137–672 aa inserted into pGEX5X-3 between *Eco*RI and *Not*I sites (Herold et al., 2001)) served as the templates for the synthesis of the radiolabeled probes referred here to as PI and AJ, respectively, using Random Primed DNA Labeling Kit (Amersham) and [α^{-32}P]ATP. The membranes were hybridized with indicated probes for 8–12 h at 68 °C using standard procedures and Rotary Hybridization Oven (Boekel Rock 'N' Roll^{TM}). Before autoradiography all blots were subjected to stringent washing conditions (0.2% SDS and 0.1× SSC at 56 °C three times for 30 min), and then were exposed to film (Kodak BIO MAX) for 24–48 h at −70 °C with an intensifying screen. RNA markers G3191 (Sigma) were used as molecular weight standards.

As the radiolabelled probes we also used the genome fragments: 7.7RS and 3.3RS subclones from 3.18.8 clone and 2.4RH and 4.0HH subclones from 3.18.7 clone, obtained by chromosome walk (Kozlova et al., 1994; Tretyakova et al., 2001).

2.4. RT-PCR analysis

RNA was extracted from heads, testes and ovaries with TRIzol Reagent (Invitrogen) following the manufacturer's protocol and was treated with DNase. RNA samples from different tissues were isolated in parallel and served as cross-controls for the potential DNA contamination. The quality of RNA preparation was checked further by visualizing RNA in agarose gel. The reverse transcription into cDNA was performed by using SuperScript III Reverse Transcriptase (Invitrogen), specific primers and 1 μl of the isolated RNA in a 20-μl reaction.

Amplification was performed at 95 °C for 10 min; 5 cycles of 94 °C for 60 s, 58 °C or 61 °C for 90 s, 72 °C for 100 s; 35 cycles of 94 °C for 40 s, 62 °C or 65 °C for 90 s, 72 °C for 90 s; and final extension at 72 °C for 10 min.

Amplicons were extracted from gel and purified using the Omnix Gel Extraction Kit (Omnix). Purified products were sequenced using the ABI BigDye Terminator Sequenced Kit v 2.1 (Applied Biosystems). Sequencing reactions were separated on an ABI 377 DNA Analyzer.

Forward primers: 5′ TGCTGAAATTCGCATATCTT 3′ (ex 1F);5′ tcggtcgctgctatctgtg (in 3–4F); 5′ ACTCTTGAGTGCTCTATTGGCAG 3′ (ex 4F-1); 5′ TGACAACTCCATTAGCAGCAT 3′ (ex 4F-2); 5′ TATGCCCTCCACTTCCAGTG 3′ (ex 9F).

Reverse primers: 5′ aatatgcggcagcatccactag 3′ (in 5–6R); 5′ CAGATAGGATGCCTTCGTT 3′ (ex 7R); 5′ AGGCGGCATGGTTAAAGTC3′ (ex 10R-1); 5′ ATTCGATTATGTGGATGTGGC 3′ (ex 10R-2); 5′ ATTGCTACTGCCCGAATGTG 3′ (ex 10R-3); 5′ TAAAGGGACTACCGTTCGCAG 3′ (ex 10R-4); 5′ ATCGCCTTGGTTTCGGTAT 3′ (ex 10R-5).

The primer sequences in introns appear in lowercase and in exon - in uppercase.

2.5. Nucleotide sequence alignments

Nucleotide sequence alignments were performed using ClustalW (http://www.ebi.ac.uk/clustalw/).

3. Results

3.1. D. melanogaster sbr gene produces several transcripts: the 2.8-kb transcript is abundant in testes and the 5.1-kb transcript is abundant in adult head

Since the mutations of *sbr* gene affect primarily the reproductive and nervous systems, we analyzed its expression in testes, ovaries and