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Dm nxf1/sbr gene affects the formation of meiotic spindle in female *Drosophila melanogaster*

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Abstract The *small bristles* (*sbr*) gene of *Drosophila melanogaster* belongs to the family of *nuclear export factor* (*NXF*) genes that participate in mRNA nuclear export. During meiosis, females of *Drosophila melanogaster* that carry various combinations of mutant alleles of the *Dm nxf1*/*sbr* gene exhibit disruption of the division spindle and misalignment of chromosomes at the metaphase plate. Meiosis of sbr^5/+ females is characterized by the formation of tripolar spindles during the first cell division. According to the sequencing results, the sbr^5 *(l(1)K4)* lethal allele is a deletion of 492 nucleotides. In SBR^5 protein, 57 of the 146 amino acids that have been lost by deletion belong to the NTF2-like domain.

Keywords *Drosophila* · female meiosis · mutant alleles · nuclear export factor · *Dm nxf1/sbr*

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Abbreviations

NPC	nuclear pore complex
NTF2	nuclear transport factor 2
NXF	nuclear export factor
RBD	RNA-binding domain
sbr	*small bristles* gene
UBA	ubiquitin-associated

Introduction

The *small bristles* (*sbr*) gene of *Drosophila melanogaster* belongs to the evolutionary conserved family of *NXF* (nuclear export factor) genes, found in many eukaryotic organisms ranging from the yeast *Saccharomyces cerevisiae* to *Homo sapiens* (Segref et al. 1997; Tan et al. 2000; Herold et al. 2003). The genes of this family are involved in controlling the transport of mRNA (Conti and Izaurralde 2001; Herold et al. 2001; Jun et al. 2001; Sasaki et al. 2005; Tretyakova et al. 2005). The *sbr* or *Dm nxf1* gene of *Drosophila melanogaster* encodes the main transport receptor of mRNA and is orthologous to the *Tap/Hs nxf1* gene of humans and the *Mm nxf1* gene of mice (Herold et al. 2001, 2003; Sasaki et al. 2005; Tretyakova et al. 2001).

Mutant alleles of the *Dm nxf1/sbr* gene in *Drosophila melanogaster* are characterized by a broad range of pleiotropic effects (Nikitina et al. 2003), which in itself is not surprising, as NFX1 transport

receptors are involved in most, if not all, types of mRNA (Herold at al. 2003). There are, however, descriptions of dominant, allele-specific effects among mutant alleles of the *sbr* gene (Nikitina et al. 2003). This allows us to assume that the function of the *Dm nxf1* gene is not limited to the nuclear transport of mRNA.

One of such allele-specific effects is the dominant role that the sbr^{10} *(l(1)ts403)* thermosensitive allele plays in affecting the frequency of aneuploid offspring of females that have been exposed to elevated temperature (Nikitina et al. 2003; Pougatcheva and Mamon 2005). Moreover, over 10% of offspring of sbr^{10}/*Df(1)L4* females (deletion of the *sbr* gene) at normal temperature exhibit disruptions in embryonic mitoses (Golubkova et al. 2006). After exposure to a heat shock, sbr^{10} mutants exhibit a significantly decreased proliferation activity of somatic cells (Mamon and Kutskova 1993). The data suggest that the *sbr* gene not only controls the nuclear transport of macromolecules but is also involved in controlling cell division. Thus, the *sbr* gene may very well become one of the genes whose products are involved in nucleocytoplasmic transport of macromolecules during the interphase and during the formation of the cell division apparatus (Nachury et al. 2001; Wiese et al. 2001; Hetzer et al. 2002; Babu et al. 2003; Mamon 2005).

NFX1 proteins have a multidomain structure (Herold et al. 2000, 2001). Functionally, SBR/dmNXF1 protein and its orthologue, TAP/Hs NXF1, can be divided into two parts. Its N-terminus is responsible for the interaction of this transport receptor with mRNA directly or via adapter molecules. The C-terminus interacts directly with nucleoporins, thus transporting RNP through the central channel of the nuclear pore complex (NPC) (Ryan and Wente 2000; Braun et al. 2001). The N-terminus of SBR is represented by an RNA-binding domain (RBD) and leucine-rich repeats. The C-terminus contains a sequence homologous to the nuclear transport factor 2 (NTF2) and called an NTF2-like domain, and a sequence that is similar to the ubiquitin-associated (UBA) domain, which is called a UBA-like domain (Herold et al. 2000). Both sequences of the C-terminus part of the protein are important for transporting the complex through the NPC (Braun et al. 2001, 2002; Fribourg et al. 2001). Functioning in macromolecular complexes, they interact with various partner proteins, including those that are involved in forming the cell division apparatus (Blevins et al. 2003; Tretyakova et al. 2005). It is, thus, important to determine the domains of SBR protein that lead to chromosome instability.

The sbr^{10} allele has been sequenced (Korey et al. 2001), and it has been demonstrated that the DNA that corresponds to this allele has significant nucleotide substitutions—9439C>T in exon 4 and 12976A>T and 12996G>T in exon 9. These substitutions result in substitutions of P139L in the RNA-linking domain, and T493S and M499I in the NTF2-like domain of SBR10 protein, respectively.

In this study we demonstrate that the sbr^{5} mutant allele is a deletion of 492 nucleotides that removes 146 amino acids in the mutant SBR^{5} protein. Of these, 57 amino acids belong to the NTF2-like domain, and the remaining amino acids to the sequence that links the NTF2-like and UBA-like domains.

Cytologic evaluation of meiosis allows the characterization of those disruptions that may lead to aneuploid offspring of the females of *Drosophila melanogaster* caused by various alleles of the *sbr* gene. We have demonstrated that during the first meiotic division, females with various combinations of mutant alleles of the *sbr* gene exhibit misalignment of the chromosome at the metaphase plate and anomalies of the meiotic spindle. In addition, among anomalous metaphases in sbr^{5}/+ females, one third of metaphases have tripolar spindles.

Materials and methods

The following *Drosophila melanogaster* strains were used in this study

1. sbr^{5} *f car / FM6l, y* sc^{8} *dm B / Dp(1;Y) y+ v+*
2. *Df(1)v-L4,* ras^{2} m^{D} */ FM6l, y* sc^{8} *dm B / Dp(1;Y) y+ v+*
3. w^{a} *B*
4. sbr^{10} *(l(1)ts403); bw; st*
5. ras^{2} sbr^{10} *v*
6. *y cv* sbr^{10}
7. *v*
8. *y*

To isolate DNA and perform further molecular analysis of the mutant sbr^{5} allele, we used heterozygous

adult females, obtained by crossing *sbr*^{10} *(l(1)ts403); bw; st* females with *sbr*^{5} *f car / Dp(1;Y) y+ v+* males.

Amplification and sequencing DNA fragments corresponding to normal and mutant alleles of the *sbr* gene

To isolate DNA from the adult fly, we employed the method of phenol–chloroform extraction with subsequent 96% ethanol precipitation. The DNA precipitate was then resuspended in water (Sambrook et al. 1989; Ashburner et al. 2005).

We chose three pairs of primers to amplify three large DNA fragments that included the main coding exons of the *sbr* gene (Table 1).

For the amplification of DNA fragments we used a reagent kit containing the Taq DNA polymerase from MBI Fermentas, Lithuania. The electrophoretic analysis of PCR products was carried out in a 1% agarose gel. Sequencing was carried out using the modified Sanger method with the help of BigDye Terminator Kit v2.0 from Perkin Elmer, Waltham, MA, USA.

For sequencing, DNA fragments were precipitated using standard ethanol precipitation with addition of a 3 mol/L solution of sodium acetate. DNA fragments were then denatured for 2 min at 95°C in formamide and TE buffer solutions, and sequenced with ABI Prism 377 automatic sequencer from Applied Biosystems, Foster City, CA, USA in a 4.5% polyacrylamide gel.

The analysis of the spectrograms was carried out using ABI Prism Seqscan v4.5 from Applied Biosystems for Mac OS X and Chromas v2.3 from Technelysium for Windows.

Table 1 Primers used to amplify three large DNA fragments that included the main coding exons of the *sbr* gene

Fragment	Strand	Sequence (5′–3′)
I	Sense 1	TGCTGAAATTCGCATATCTT
	Antisense 3	aaactcacCGTGACTTGGTA
II	Sense 4	ACTCTTGAGTGCTCTATTGGCAG
	Antisense 5	aatatgcggcagcatccactag
III	Sense 6	atgtccactatttacagCGAAG
	Antisense 10	TAAAGGGACTACCGTTCGCAG

Sequences that correspond to exons are in capital letters, introns in lowercase letters, and nontranslatable in bold type.

Prediction of the secondary structure of SBR proteins

Predictions for the secondary structure of SBR proteins were carried out using the SWISS-MODEL modeling server (swissmodel.expasy.org/workspace).

Cytological analysis of meiotic divisions in *D. melanogaster* females

For the cytological analysis of meiotic divisions we used females resulting from crossing the aforementioned strains. Three-day-old virgin females were crossed *en masse* with *w*^{a} *B* males in a 1♀:1♂ ratio. In the next two days, ovaries of the females were used for preparations using the method described by Nokkala and Nokkala (2003).

Results

The nature of the *l(1)K4 (sbr*^{5}*)* deletion mutation separated using analysis of the size of DNA fragments obtained using PCR

In order to understand the molecular nature of the *sbr*^{5} mutant allele using the PCR method, we amplified and then separated by gel electrophoresis three sections of the *sbr* gene. For this, we matched primers with three exon blocks 1–3, 4–5, and 6–10, separated by introns 3–4 and 5–6 with lengths of 8892 bp and 1602 bp, respectively (see Fig. 1). For PCR we used DNA extracted from *sbr*^{10} *(l(1)ts403); bw; st* females and *sbr*^{5}*/sbr*^{10} females. Since the *sbr*^{5} is a lethal allele, the molecular analysis of the sequence that corresponds to this allele is possible only when using heterozygous specimens. To obtain DNA we took *sbr*^{5}*/sbr*^{10} heterozygotes, since the *sbr*^{10} *(l(1)ts403)* allele is characterized by a series of point substitutions (Korey et al. 2001) and is different from the sequence of the wild type allele found in the Ensembl database (www.ensembl.org). We have observed such single-nucleotide substitutions in all the amplified DNA fragments.

Sequencing of the first fragment has demonstrated that the sequence corresponding to the *sbr*^{10} allele has the C531T neutral substitution in the encoding section of exon 1, compared with the sequence found in the Ensembl database. For the *sbr*^{5} allele we found the substitution of the G670T nucleotide in exon 2.

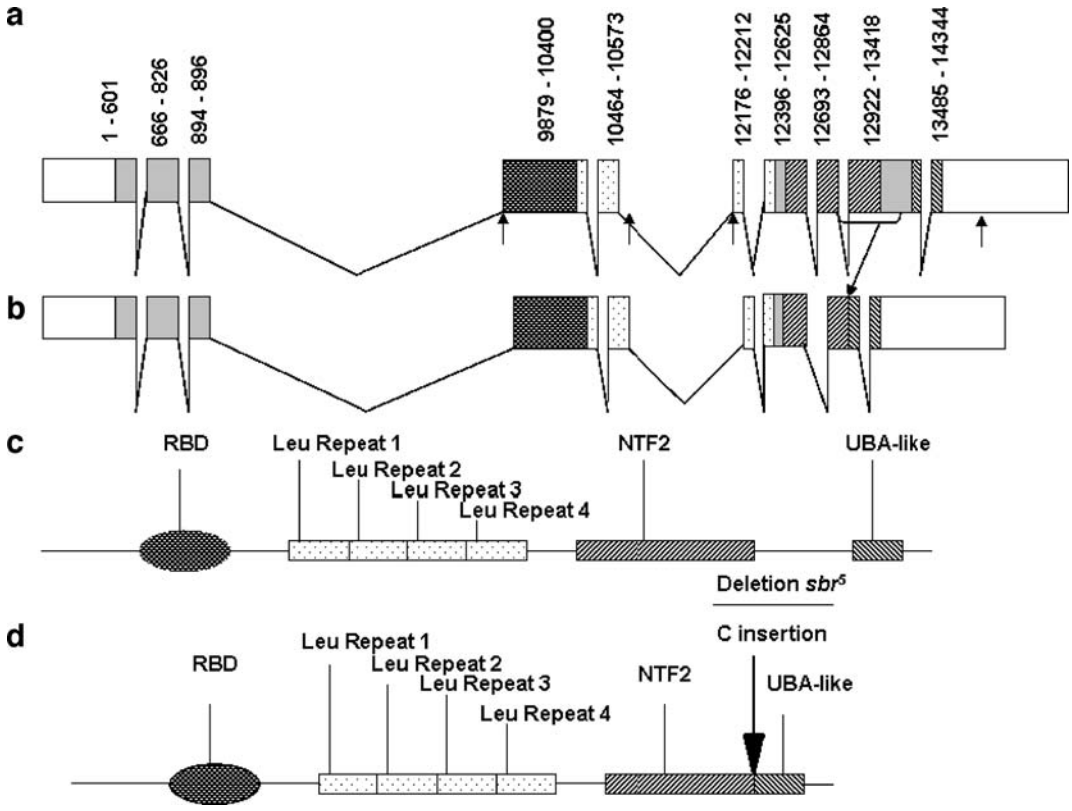

Fig. 1 Intron–exon structure of the *small bristles* (*sbr*) gene and domain organization of SBR protein in *D. melanogaster*; **(a, c)** normal; **(b, d)** lethal sbr^5 allele. Legend: ↑ attached primers ⌊⌋ deleted segment of the gene ▭ linker segments of SBR protein Domains: ▩ RBD (RNA-binding domain) ⁘ LLR (leucine-rich repeats) ▨ NTF2-like (nuclear transport factor 2-like) domain ▨ UBA-like (uniquitin-associated-like) domain

For the second sequenced fragment, which includes exons 4 and 5 and intron 4–5, we separated the significant substitutions C9943T in exon 4, as described earlier for the sbr^{10} allele (Korey et al. 2001). It substitutes proline in position 139 for leucine (P139L) in SBR^{10} mutant protein in the RNA-binding domain. The sequence of segment 2 that corresponds to the sbr^5 allele does not differ from that found in the Ensembl database.

The third fragment for each allele was sequenced separately, for, as we have demonstrated, the sequence corresponding to the sbr^5 allele contains a deletion, whereas the size of fragments 1 and 2 is identical for both alleles that we have studied (see Fig. 2a and b).

A comparison of section 3 has demonstrated that the DNA sequence that corresponds to the sbr^{10} allele differs from that found in the Ensembl database. In section 3 the DNA corresponding to this allele has

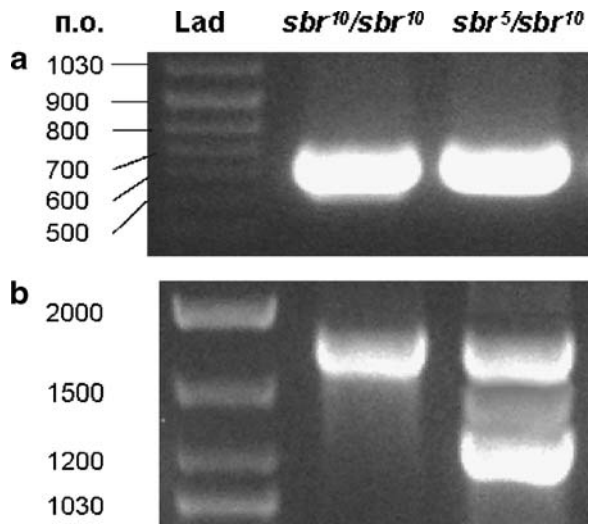

Fig. 2 Results of PCR amplification of segments of the *sbr* gene of *D. melanogaster* containing **a** exons 4 and 5 and an intron between them, and **b** exons 6, 7, 8, 9, and 10 and introns between them

significant nucleotide substitutions 12976A>T and 12996G>T, which lead to substitutions T493S and M499I in SBR10 protein, respectively, as was already known (Korey et al. 2001) and as we have demonstrated. We have also demonstrated that intron 6–7 has four single nucleotide substitutions and one deletion the size of two nucleotides. Exon 7 has two neutral nucleotide substitutions—12491T>C and 12599A>G.

The DNA sequence that corresponds to the sbr^5 allele contains a deletion of 492 nucleotides that includes exons 8 and 9. This deletion removes five flank nucleotides from exon 8, the entire intron 8–9 of 58 nucleotides, and 430 nucleotides from exon 9. This results in the formation of a chimeric exon 8/9 with the 13858C>T substitution in the break point (Fig. 3). At the same time, the reading frame is preserved. In addition, the DNA sequence that corresponds to the sbr^5 allele differs from that found in the Ensembl database—in the following substitutions: A(12264)C, G(12288)T, T(12292)A, C(12387)T, T(12491)C, A (12599)G, C(12858)T—and carries the deletion of two nucleotides (CA) −2dell2294.

Thus, in the predicted protein product of the sbr^5 mutant allele, compared to normal SBR protein, amino acids 473 through 618 have been removed and replaced with the amino acid cysteine. At the same time, 57 of the 146 deleted amino acids belong to the NTF2-like domain, while the remaining amino acids belong to the section that links the NTF2-like and UBA-like domains (Fig. 1). The prediction of secondary structure of proteins encoded by *sbr* alleles suggested that changes in SBR^5 protein structure occur not only in the NTF2-like domain but also in the N-terminus domain, whereas the UBA-like domain remains unchanged (Fig. 4). For the product of the sbr^{10} allele, SBR^{10}, the change in the NTF2-like domain was evident (Fig. 4).

Molecular analysis of the sbr^5 allele shows that, in addition to the full-size SBR protein, sbr^5/sbr^{10} heterozygotes have a small-size protein SBR^5. The finding that the fecundity of sbr^5/sbr^{10} females (the number of female offspring per one parent female per day) is significantly greater than the fecundity of $sbr^{10}/Df(1)v\text{-}L4$ ($sbr^{10}/0$) females that are heterozygous with the null allele of the *sbr* gene (Fig. 5) testifies to the fact that the allele sbr^5 is not a null allele. In addition, dominant allele-specific effects have been observed in the sbr^5 allele—disruption of meiosis, which is characteristic of $sbr^5/+$ and sbr^5/sbr^{10} females.

Cytological analysis of meiotic deletions in females

One of the characteristics of the sbr^{10} thermosensitive allele is the dominant effect of the mutation of sbr^{10} on sex chromosome segregation during meiosis in females, which has been demonstrated by a hybridological analysis (Nikitina et al. 2003). In this respect, the sbr^{10} allele exhibits gain-of-function mutations, since disruptions of the chromosome segregation is evident only in $sbr^{10}/+$ and sbr^{10}/sbr^{10} females, and not in females hemizygous with the *Df(1) v -L4/+* wild type allele. Thus, it is the very presence of mutant SBR10 protein, and not the reduction of the dose of normal protein (such as is observed in *Df(1) v -L4/+* females), that leads to the disruption of chromosome segregation.

A cytological study of meiosis in females of *D. melanogaster*, which carry various combinations of the *sbr* gene, reveals the disruption that may lead to aneuploid offspring (Fig. 6).

Results of the analysis of preparations have shown that sbr^{10}, sbr^5/sbr^{10}, $+/sbr^5$ females, and especially $0/sbr^{10}$ females are characterized by a high frequency of disruption of the first meiotic division (Table 2). $0/sbr^{10}$ females exhibit the highest frequency of disruption, reaching 73% (Table 2). sbr^5/sbr^{10} females have a lower frequency of meiotic disruption, at 38.5%, while the frequency of disruption in females homozygous with the sbr^{10}/sbr^{10} mutation is 17.9% (Table 2). It is interesting to note that females with the mutant sbr^5 allele in combination with a wild-type allele also exhibit a higher frequency of anomalous metaphases of the first meiotic division, 43.4%.

The presence of the wild-type allele of the *sbr* gene in a single dose does not affect the frequency of anomalies in the first meiotic division, where a single dose of the sbr^{10} allele increases the frequency of disruption by several times (Table 2). Since the frequency of disruption of meiosis in sbr^5/sbr^{10} females is significantly lower than that of $0/sbr^{10}$ females, it is safe to assume that the sbr^5 allele is not a null allele. Moreover, the presence of the sbr^5 allele causes characteristic meiotic disruptions, which also signals the functionality of its corresponding mutant product.

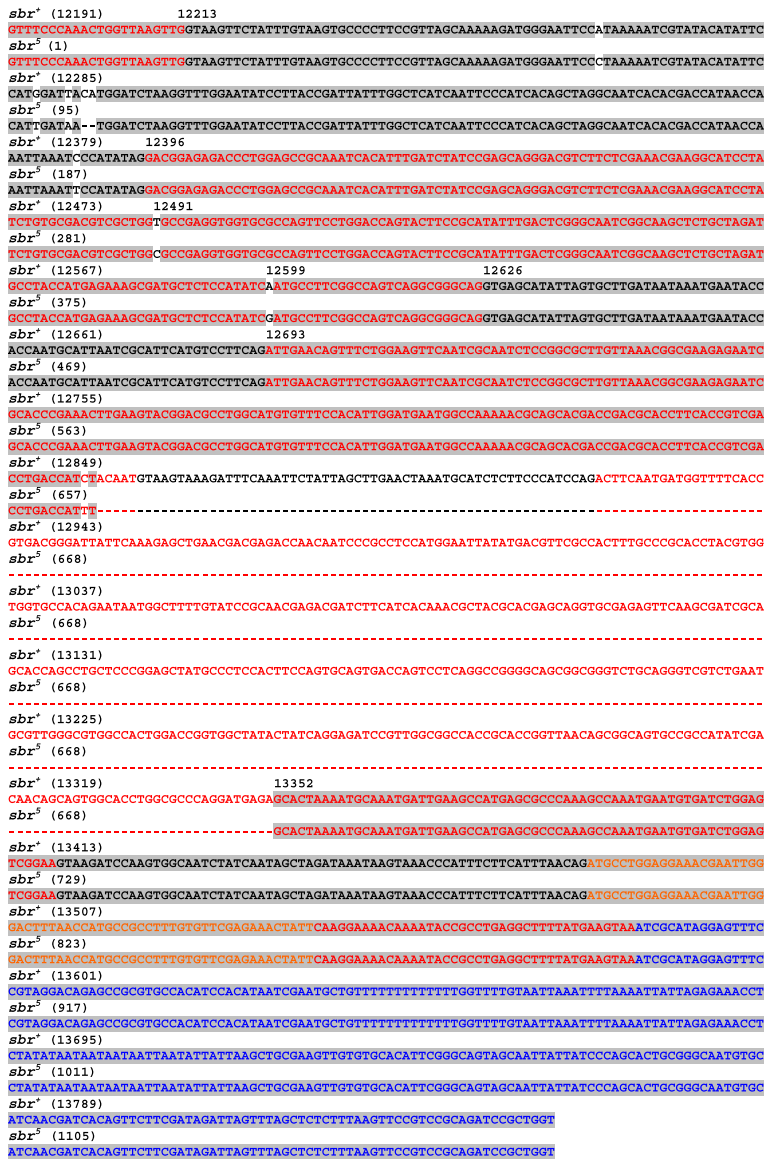

Fig. 3 Results of the alignment of the nucleotide sequence of exons 6–10 for sbr^{+} wild-type alleles and sbr^{5} lethal alleles. Red letters denote segments of exons; black letters represent introns; blue letters represent nontranslatable segment of exon 10. Nucleotides that are identical in the compared sequences are marked in gray. For the *sbr* + allele, the nucleotides are numbered starting at the beginning of the transcript, in accordance with the sequence found in the Ensembl database, and for the sbr^{5} allele starting from the beginning of the sequence fragment

Figure 7 depicts a spectrum of various types of disruptions during the first meiotic division in females with statistically significant increase of the frequency of anomalous metaphases. If we look at the disruptions in metaphase I, which are characteristic of the females carrying the sbr^{10} allele but having no wild-type allele, we see that half of the anomalous metaphases demonstrate changes in the shape of the meiotic spindle and a characteristic disruption of separation of bivalents at the metaphase plate (see Fig. 8b, c). Often, bivalents are not separated at all and form a single mass of chromatin (Fig. 8b). On the other hand, sbr^{5}/+ females exhibit only 7% of the disruptions of this class, and among anomalous metaphases tripolar metaphases prevail (around 30%) (Fig. 7; Fig. 8d). Tripolar metaphases are an anomaly characteristic only of sbr^{5}/+ females. Females with the sbr^{10} allele do not exhibit such disruptions. Among metaphases with a misshaped spindle and disrupted separation of bivalents, $sbr^{10}/0$ females demonstrate metaphases with a reduced number of bivalents (Fig. 8c). Metaphases I with a reduced number of bivalents may indicate disruptions in chromosomal segregation during preceding mitoses.

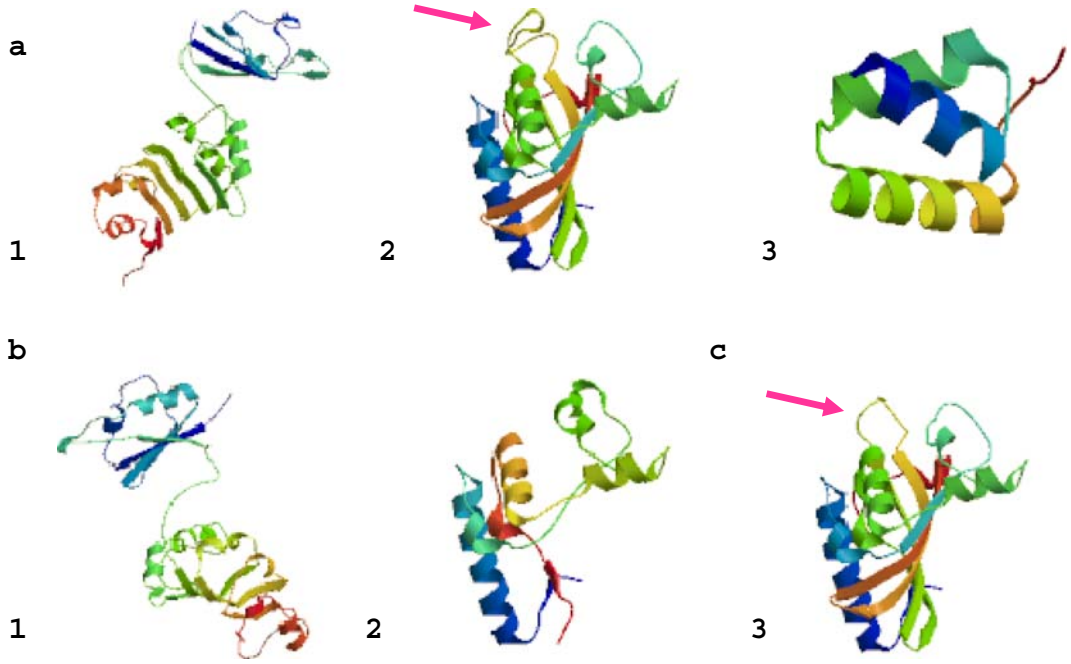

Fig. 4 Prediction for the secondary structure carried out using the SWISS-PROT modeling server (swissmodel.expasy.org/workspace). **a** Product of a wild-type allele: 1, RNA binding domain; 2, NTF2-like domain; 3, UBA-like domain. **b** Product of the *sbr*^{5} allele: 1, RNA binding domain; 2, NTF2-like domain. **c** Product of the *sbr*^{10} allele: 2, NTF2-like domain

Discussion

Mutant alleles of the *sbr* gene disrupt the formation of the meiotic spindle in females of *D. melanogaster*

One of the pleiotropic effects of mutant alleles of the *sbr* gene is an increased frequency of aneuploid offspring (Mamon et al. 1990; Nikitina et al. 2003; K'ergaard and Mamon 2007), which leads us to assume that the product of this gene participates in forming the cell division apparatus. The mechanisms of this effect, however, are unknown. In this study we have demonstrated which meiotic disruptions are caused by the *sbr*^{5} and *sbr*^{10} mutant alleles (Fig. 7), and found allele-specific disruptions, which allow us to make an assumption of the functional significance of the sections of Dm NXF1 protein that undergo changes as a result of mutation. The *sbr*^{5} deletion

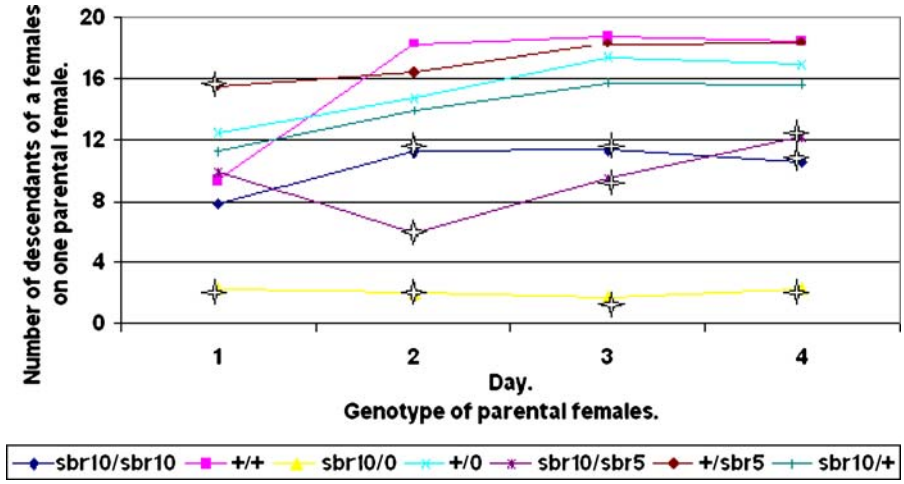

Fig. 5 Fecundity of females of different genotypes under control, determined every 24 h over a period of four days in a row ✧ denotes data statistically different ($p<0.05$) from those for +/+ females

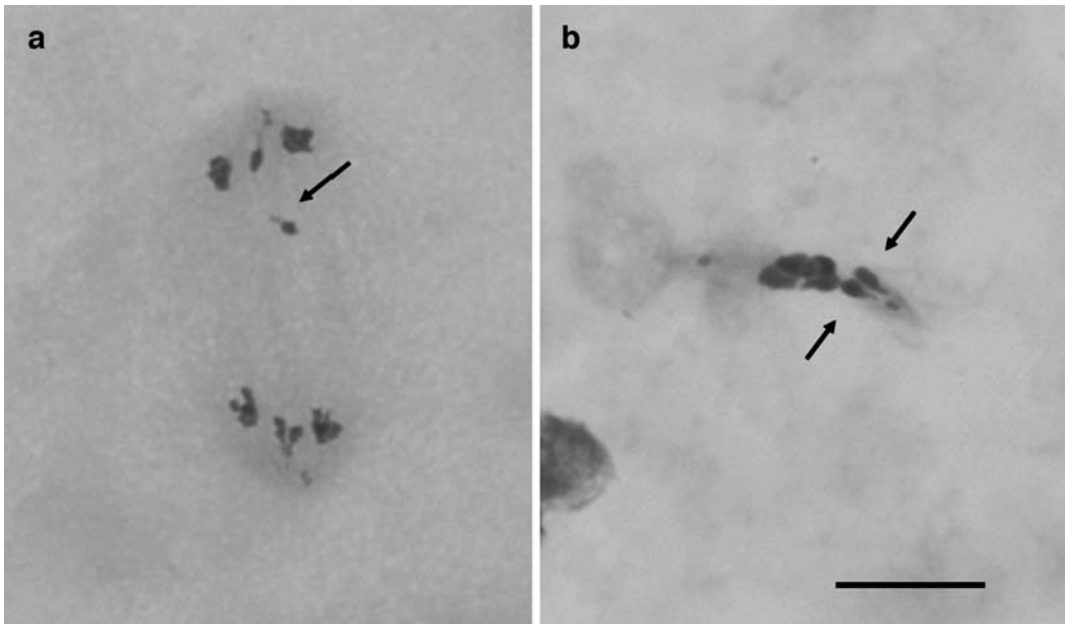

Fig. 6 Cytological demonstration of nonsegregations and losses of chromosomes in meiosis in females with the sbr^{10} allele. **a** Loss of one arm of the X chromosome. **b** Nonsegregation of sex chromosomes (both X chromosomes migrate toward the same pole). (Bar represents 5 μm)

affects the C-terminus of SBR protein, i.e., the region of orthologue proteins, which is responsible for binding them with nucleoporins (Bachi et al. 2000; Izaurralde 2001; Grant et al. 2002). The deletion resulted in the removal of a portion of the NTF2-like domain and a flexible linker that connected the NTF2-like domain with the UBA-like domain (Fig. 1).

Table 2 Frequency of disruptions during the first meiotic division in females of *Drosophila melanogaster* carrying various combinations of the alleles of the *sbr* gene

Female genotype	Frequency of anomalous phases (%)	Sampling (number of analyzed metaphase plates)
$0/sbr^{10}$	73.3 ± 4.3^{abc}	105
sbr^5/sbr^{10}	38.5 ± 5.1^{ab}	91
+/+	1.7±1.2	115
sbr^{10}/sbr^{10}	17.9 ± 3.6^{ad}	112
$sbr^{10}/+$	4.5±2.2	89
$sbr^5/+$	43.4 ± 5.0^{a}	99
0/+	3.2±1.8	95

Differences are statistically significant at $p<0.05$: [a] between +/+ females and other studied females; [b] between $0/sbr^{10}$ and sbr^5/sbr^{10} females; [c] between $0/sbr^{10}$ and other studied females; [d] between sbr^{10}/sbr^{10} and other studied females.

Changes in protein structure may affect its interaction with its partner proteins. It is known that NXF1 proteins function as a part of macromolecular complexes and interact with different factors (Kataoka et al. 2000; Stutz et al. 2000; Le Hir et al. 2001; Rodrigues et al. 2001; Gatfield and Izaurralde 2002; Blevins et al. 2003; Huang et al. 2003; Forler et al. 2004; Ooe et al. 2004; Tretyakova et al. 2005). Such interactions have been demonstrated for orthologues of Dm NXF1 in humans (Hs NXF1/TAP) and mice (Mm NXF1). Experimental evidence of interactions of Dm NXF1 protein with other proteins is limited (Herold et al. 2001).

There are no data so far on the interaction of Dm NXF1 protein or its orthologues with the cell division apparatus or chromosomes, but there are known factors among partner proteins of NXF1: Gle2/Rae1 (Bachi et al. 2000) and MAP1B and MAP1A (microtubule associated protein) (Tretyakova et al. 2005). It has been demonstrated that the C-terminus of TAP/Hs NXF1 protein (Bachi et al. 2000; Blevins et al. 2003) is responsible for the interaction with Rae1 protein, which is involved in cell division. Rae1 is necessary for the assembly of the division spindle, since removing this protein from extracts of *Xenopus* eggs suppresses spindle assembly, and reducing the

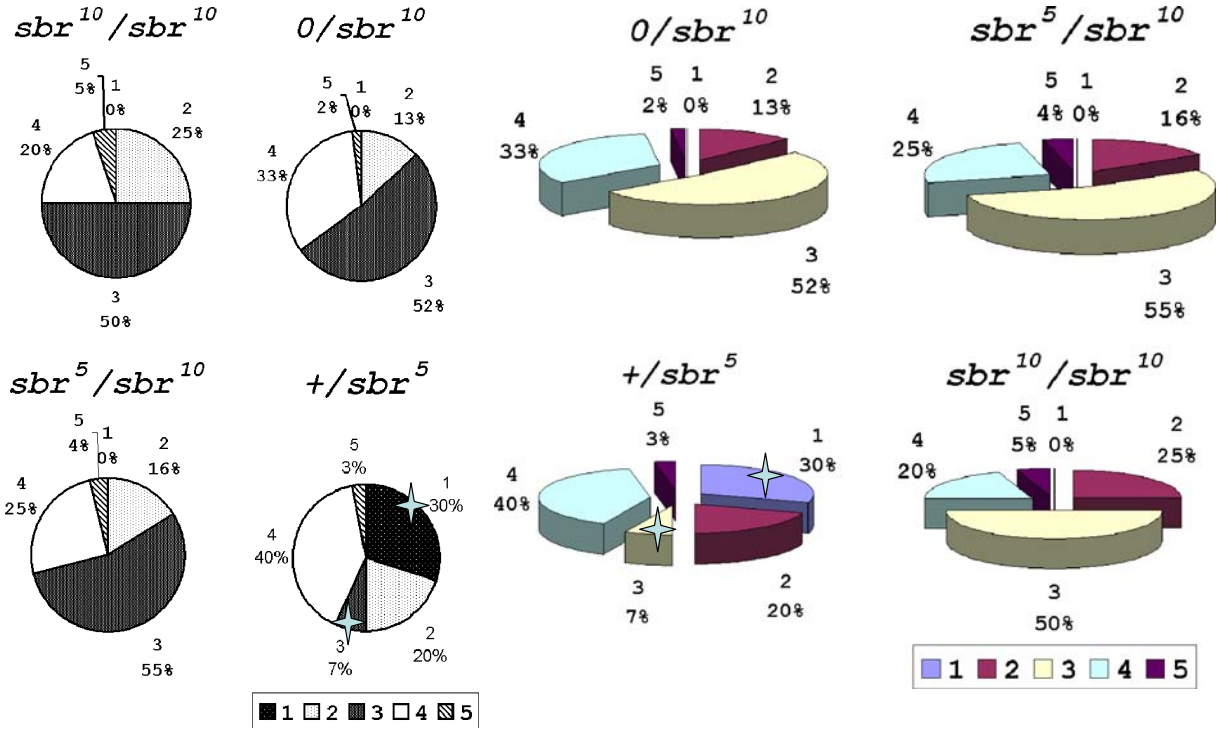

Fig. 7 Different types of disruption of the first meiotic division in females of *Drosophila melanogaster* with statistically significant high frequency of deviations from the norm: 1, tripolar meioses; 2, metaphases with misshaped spindle; 3 disruption of individualization of bivalents; 4, losses and nonsegregations of chromosomes; 5 other disruptions ✦ denotes data statistically different ($p<0.05$) from those for females of other studied genotypes

activity of Rae1 using the siRNA *Rae1* method in HeLa cells exhibits the formation of tripolar spindles (Blower et al. 2005; Wong et al. 2006). The Rae1 factor is an evolutionarily conserved protein, and in extracts of *Xenopus* eggs it exists in the form of RNP complexes. The RNA that is part of these complexes is a mandatory component for the construction of the division spindle (Blower et al. 2005).

The Dm Rae1 protein in *D. melanogaster* is 67% homologous to the orthologous Hs Rae1 protein in *H. sapiens*. It is also important for cell proliferation and the cell's entry into S phase after G_1 stage of the cell cycle (Sitterlin 2004). Being part of RNP complexes, the Rae1 factor interacts with other proteins bound with mRNA, such as Maskin, which also interacts with microtubule (Groisman et al. 2000). TACC3, which is the Maskin homologue in humans, functions in spindle assembly in culture cells (Gergely et al. 2003), and the *d-tacc*^{1} mutation in the homologous gene in *D. melanogaster* leads to the formation of tripolar meiotic spindles in females (Cullen and Ohkura 2001).

It should be noted that both the single-nucleotide substitutions in exon 9 that lead to substitutions of amino acids T493S and M499I in SBR^{10} protein are localized in the region that has been deleted in SBR^{5} protein. It is these substitutions that affect the supposed structure of the protein molecule (Fig. 4). The role that these substitutions play in protein interactions is unknown.

The data that we have obtained allow us to assume that the C-terminus of Dm NXF1 protein has the potential for cytoplasmatic functions that may have to do with the formation of the meiotic spindle in females of *D. melanogaster*.

Tripolar spindles during meiosis in *sbr*^{5}/+ females and factors that participate in forming spindle pole bodies as possible partners of Dm NXF1/SBR protein

The high frequency of tripolar metaphases in *sbr*^{5}/+ females allows us to assume that the Dm NXF1 protein is necessary not only for enabling contact of the plus-tips of microtubules with the chromosomes, but

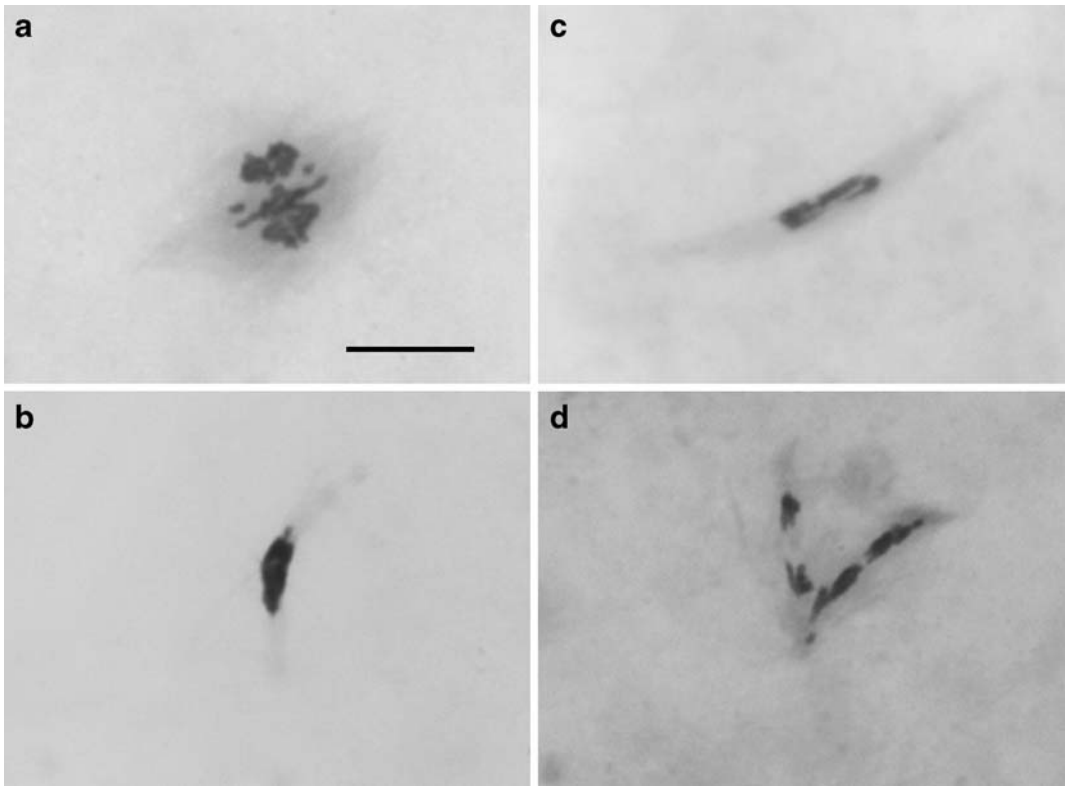

Fig. 8 Metaphase of the first meiotic division of females of *Drosophila melanogaster*. **a** Normal metaphase of the first meiotic division of a +/+ female. **b** and **c** Anomalous metaphases of the first meiotic division of a $sbr^{10}/0$ female; **b** misshapen spindle, disrupted bivalent individuality; **c** narrowed spindle, lower number of bivalents. **d** Tripolar metaphase of the first meiotic division of a $+/sbr^{5}$ female (Bar represents 5 μm)

also for forming spindle pole bodies. It is even possible to determine the area of the protein molecules that plays a special role in the process of focusing microtubules on the spindle pole bodies: it is the area that includes a portion of the NTF2-like domain and the area of the flexible linker that binds the NTF2-like domain with the UBA-like domain, and is absent in the mutant SBR^{5} protein. It is noteworthy that the formation of tripolar spindles in meiosis I is observed only if the mutant SBR^{5} protein is present alongside the normal SBR protein, i.e., in heterozygous $sbr^{5}/+$ females. In addition, in $sbr^{5}/+$ females metaphases without a spindle have been observed (no data shown here).

At first, the absence of tripolar spindles in meiosis I in sbr^{10}/sbr^{5} females may seem paradoxical. If, however, we take into account the differences in the disruption of individualization of chromosomes on the metaphase plate in $sbr^{5}/+$ and sbr^{5}/sbr^{10} females, we can assume that the formation of tripolar spindles takes place only if the portion of disruption of individualization of chromosomes at the metaphase plate is small (about 7%).

The focusing of microtubules takes place as a result of the sliding of macromolecular complexes toward the minus ends of the microtubules, which cross-links them (Dammerman et al. 2003). The proteins D-TACC, Msps, and Ncd participate in focusing microtubules on the pole bodies (Matthies et al. 1996; Cullen and Ohkura 2001). Functional disruptions of either protein may lead to the formation of additional spindle pole bodies (Kwon and Scholey 2004). It has been demonstrated that to form acentrosomic meiotic spindles in females of *D. melanogaster*, the complex comprising D-TACC, Msps, and Ncd must be active. Mutants of any of these three genes lead to the formation of tripolar spindles or the disruption of focus in spindle formation (Matthies et al. 1996; Cullen and Ohkura, 2001).

Peculiarities of meiosis in females of *D. melanogaster*

A distinct feature of meiosis in females of many living organisms, including *D. melanogaster*, is the fact that the meiotic spindle forms in the absence of centrosomes and astral microtubule arrays (Compton 2000; Karsenti and Vernos 2001). Chromosomes are functioning as the centers of the nucleation of the microtubule arrays. As the arrays grow, their focus shifts toward spindle pole bodies (McKim and Hawley 1995). Short microtubule arrays already separate from the chromosomes before the formation of the first meiotic spindle in *D. melanogaster* (Hatsumi and Endow 1992). The formation of the anastral spindle in *D. melanogaster* takes place in several stages. Stage one occurs when the nuclear envelope breaks down and microtubules interact with the chromosomes (Theurkauf and Hawley 1992; Skold et al. 2005). It is possible that this interaction stimulates the individualization of bivalents and turns the compact mass of chromatin into discrete bivalents easily identifiable on the metaphase plate. The next stage in the formation of the meiotic spindle is the process of alignment of the bivalents along the midzone of the metaphase plate. This process occurs with the participation of the microtubules that have been focused on the spindle pole bodies (Theurkauf and Hawley 1992; Heald et al. 1996). In *sbr* gene mutants, both processes—the individualization of chromosome bivalents at the metaphase plate and their alignment along the spindle midzone—are disrupted (Fig. 8b, c). In addition, the first meiotic spindle in mutant females is narrower than that in females that have at least one wild-type allele. Since the anomalies of the first meiotic division that we describe in this paper are connected foremost with the disruption of the meiotic spindle, it is important to understand at which stages of the formation of the meiotic spindle (nucleation of microtubules, growth and stabilization of microtubule tips, and the formation of cross-links between microtubules, which leads to their focusing on the spindle pole bodies) the participation of the SBR protein is necessary. It is possible that Dm NXF1 protein is part of the complexes that enable the contact of microtubules with chromosomes during the first stage of forming the meiotic spindle in *D. melanogaster*. This reduces the number of microtubules that contact with the chromosomes in mutants, and the spindle looks narrower.

Conclusion

Because the NXF1 factor is an RNA transport receptor, it is particularly important to study the role of RNA complexes in spindle assembly (Mamon 2008). Experiments have shown that the presence of RNA is necessary for spindle assembly (Blower et al. 2005; Funabiki, 2005). Moreover, the Rae1 factor, which interacts with Hs NXF1/TAP, is also part of the complexes containing RNA (Bachi et al. 2000; Blevins et al. 2003). The C-terminus of the NXF1 molecule is essential for this interaction (Bachi et al. 2000). At the same time, the Rae1 factor interacts with Maskin protein (orthologue of D-TACC), which binds with both mRNA and microtubules (Groisman et al. 2000). It is possible that the DmNXF1 factor, which participates not only in the nucleocytoplasmic transport of mRNA (Herold et al. 2001) but also in forming the cell division apparatus, is also part of such mRNA complexes.

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