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RESEARCH ARTICLE

A specific SR protein binds preferentially to the secretory protein gene transcripts in salivary glands of *Chironomus tentans*

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Abstract The members of the serine–arginine (SR) family of proteins play multiple roles in posttranscriptional gene expression. Initially considered as essential splicing factors confined to the nucleus and regulating constitutive and alternative splicing, SR proteins are now known to shuttle between the nucleus and the cytoplasm and to be involved in mRNA biogenesis, transport, and translation. In *Chironomus tentans*, hrp45 is an SR protein structurally similar to the *Drosophila* SRp55/B52 SR protein. We have studied how hrp45, hrp36 [a heterogenous nuclear ribonucleoprotein (hnRNP) protein], and small nuclear RNP (snRNP) proteins are distributed in the transcriptionally active loci of polytene chromosomes in *C. tentans*. Immunofluorescence visualization of the proteins in double-labeling experiments revealed that hrp45 preferentially associates with a small number of puffs. On the other hand, hrp36 and snRNP proteins were found distributed in a large number of loci with little quantitative difference. Remarkably, hrp45-labeled loci coincide with the sites of transcription of premessenger RNPs of secretory protein (sp) genes. Because the labeling was found sensitive to RNase A treatment, we conclude that the SR protein hrp45 preferentially binds to sp gene transcripts in salivary gland cells. The preferential association of a specific SR protein with a particular type of gene transcripts reflects substrate-specific function(s) of an SR protein, in vivo. The possible roles that hrp45 might be playing in speedy and efficient processing of sp gene transcripts are discussed.

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Introduction

Eukaryotic mRNA is associated with a large number of proteins and form messenger ribonucleoprotein (mRNP) complexes from its synthesis in the cell nucleus to its translation into proteins in the cytoplasm. The proteins in the mRNP complexes can accompany the RNA from gene to polysomes or just be associated with the transcript during a specific phase in mRNA biogenesis (for reviews, see Daneholt 2001; Dreyfuss et al. 2002). Some important proteins that associate with mRNA are heterogenous nuclear RNP (hnRNP) proteins, small nuclear RNP (snRNP) proteins, and serine–arginine (SR) proteins (for review, see Reed and Magni 2001). There are more than 30 hnRNP proteins in mammalian cells that bind pre-mRNA concomitant with transcription (for review, see Dreyfuss et al. 1993; Krecic and Swanson 1999) and together form a 5- to 10-nm-thick RNP fibril for further packing into RNP particles (Fakan 1994). The snRNP proteins are components of the splicing machinery, the spliceosome, and accumulate on pre-mRNA for removal of introns (Will and Lührmann 2001).

The SR proteins are a family of evolutionarily conserved proteins that have essential roles in both constitutive and regulated splicing (reviewed in Sanford et al. 2003; Bourgeois et al. 2004). The SR proteins are

characterized by one or two N-terminal RNA recognition motifs (RRMs) and a C-terminal RS domain, rich in SR dipeptides. In vitro, SR proteins have redundant functions in splicing. However, it was also shown that SR proteins have unique functions in splicing, both in vitro (Kim et al. 1992; Zahler et al. 1993) and in vivo (Wang et al. 1996; Li et al. 2005).

Some SR proteins, such as ASF/SF2, 9G8, and SRp20, shuttle continuously between the nucleus and the cytoplasm and thus have been implicated in roles both in the nucleus and the cytoplasm (Cáceres et al. 1998). The SRp20 and 9G8 promote export of intronless mRNA in both mammalian cells and *Xenopus* oocytes (Huang and Steitz 2001). Some shuttling SR proteins act as adapters for TAP/NXF1-dependent mRNA export receptors (Huang et al. 2003). Antibodies against SR proteins inhibit the export of RNA in *Xenopus* oocytes (Masuyama et al. 2004). Furthermore, ASF/SF2 controls the stability of a PKCI-1-related mRNA (Lemaire et al. 2002) and stimulates translation both in vivo and in vitro (Sanford et al. 2004). SR proteins were also found involved in mRNA surveillance (Zhang and Krainer 2004).

The SR proteins SRp55/B52 in *Drosophila* and rsp-3 in *Caenorhabditis elegans* are essential for normal development and viability (for review, see Sanford et al. 2005). The ASF/SF2 and SC35 are required for cell viability (Lin et al. 2005). In a recent study, Li et al. (2005) reported that correct level of ASF/SF2 in cells is essential for progression of the cell cycle. Finally, Li and Manley (2005) found that ASF/SF2 is required for maintenance of genome stability.

The SR proteins are recruited from the nuclear speckles to growing pre-mRNAs at active genes (Lamond and Spector 2003). Studies on lampbrush chromosome loops in amphibian oocytes (Roth et al. 1990) and on polytene chromosome puffs of dipteran larval salivary gland (Champlin et al. 1991; Baurén et al. 1996) suggest that SR proteins are associated with a large number of nascent transcripts. However, it is not known if individual SR proteins bind to most or all pre-mRNAs and perform general functions or associate with some specificity to particular transcripts and selectively influence their expression, in vivo.

The *C. tentans* hrp45 is an SR protein of 45 kD characterized by two amino terminal RRMs and a carboxy terminal SR-rich domain. The hrp45 is structurally similar to the *Drosophila* SRp55/B52 protein (Alzhanova-Ericsson et al. 1996). Immunoelectron microscopy showed that hrp45 is added onto Balbiani ring (BR) transcripts concomitant with transcription. Recently, it was found that hrp45 is recruited to the growing BR pre-mRNAs in association with *C. tentans*-repressor splicing factor (Ct-RSF), which represses the splicing activity of hrp45 (Björk et al. 2006).

In view of the increasing information on diversified roles of SR proteins in posttranscriptional gene regulation, it is desirable to study their association with different transcripts to reveal the roles that they might be playing in the expression of specific genes. The genes in polytene chromosomes of dipteran larvae have provided excellent material for studies of the assembly and transport of pre-mRNP particles (Singh et al. 1999; Daneholt 2001). Furthermore, the polytene chromosomes display hundreds of transcriptionally active loci, the puffs, which are the morphological manifestation of unfolded active genes with RNA polymerases and nascent mRNPs.

We have studied the distribution of the SR protein hrp45 in relation to hrp36 and snRNP proteins in transcriptionally active loci of polytene chromosomes of *C. tentans* by immunofluorescence. The hrp36 is an abundant hnRNP protein in *C. tentans* homologous to human hnRNP A1 protein (Visa et al. 1996). We show that hrp45 binds preferentially to the secretory protein (sp) gene transcripts. We suggest that hrp45 plays an important role not only in splicing but also in other events in mRNA biogenesis. As this SR protein is preferentially associated with primary transcripts of sp, it might be important for speedy and efficient processing and transport of the associated transcripts during the larval stage of development when their ultimate protein product, the sp, are highly needed.

Materials and methods

Materials

The *C. tentans* was reared following the method of Lezzi et al. (1981). The fourth instar larvae were used to prepare salivary gland polytene chromosomes for immunofluorescence. *C. tentans* tissue culture cells were cultivated as a vesicle suspension at 24°C as described by Wyss (1982).

Antibodies

The mouse monoclonal antibodies (mAbs) 2E4 and 4F9 against *C. tentans* SR protein hrp45 and hnRNP protein hrp36, respectively, were raised in our laboratory by Wurtz et al. (1996). The mAb Y12 (Lerner et al. 1981) against the Sm epitope of snRNP proteins was given by Dr. J. A. Steitz, whereas mAb 4G3 (Habets et al. 1989) against the snRNP U2B″ was procured from Euro-Diagnostica B. V. (Apeldoorn, The Netherlands). Fluorescene-conjugated antibody against bromodeoxyuridine (anti-BrdU)-fluorescein isothiocyanate (FITC), which also recognizes bromouridine (BrUTP) with high specificity and affinity (Schuttle et al. 1987), was obtained from Boehringer Mannheim, Germany. The anti-human von Willebrand factor VIII antibody used

in negative control experiments was purchased from Dakopatts AB, Sweden (DAKO). All antibodies, except 4G3 and anti-von Willebrand factor VIII used in the present study, were either biotinylated and visualized by Texas Red-Streptavidin or labeled with FITC for direct detection. The unlabeled antibodies were detected by tetrarhodamine isothiocyanate (TRITC)-conjugated secondary antibody (goat anti-mouse IgG-TRITC) purchased from Boehringer Mannheim, Germany.

Direct labeling of mAbs

For immunofluorescence analyses of the simultaneous distribution of two proteins in double-labeling experiments, mAbs were directly labeled with either fluorescein or biotin using the Fluorescein Labeling Kit and the Biotin Labeling Kit, respectively (Boehringer Mannheim, Germany). The labeling reactions were carried out essentially following the procedure recommended by the manufacturer. The molar mixtures were 1:10 and the reactions were allowed to proceed for 3 h at room temperature. The labeled antibodies were separated from the nonreacted reagents by gel filtration on Sephadex G-25 columns.

SDS-PAGE and Western blot analysis

The proteins in nuclear extract (prepared as described by Wurtz et al. 1996) were precipitated with cold acetone, resuspended in sample buffer [10% glycerol, 2% sodium dodecyl sulfate (SDS), 10 mM dithiothreitol, 0.02% bromophenol blue, and 0.0625 M Tris–HCl at pH 6.8], and separated by electrophoresis in discontinuous gels containing 10% polyacrylamide and 0.1% SDS in the separating gel (Laemmli 1970). After electrophoresis, the proteins were blotted to transfer membranes (Immobilon PVDF, Millipore) using a Trans-Blot semidry electrophoretic system (BioRad, Sweden). Membranes were blocked in phosphate-buffered saline (PBS) containing 10% dry milk powder for 1 h and incubated with primary antibodies diluted in PBS containing 1% dry milk powder and 0.05% Tween-20. Labeling was visualized with alkaline phosphatase-conjugated anti-mouse immunoglobulins (DAKO) using the nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate system (Promega).

Immunostaining of polytene chromosomes

Isolated salivary glands were fixed in 2% paraformaldehyde for about 20 min and squashed in 45% acetic acid. The squash preparations were frozen over liquid nitrogen, and thereafter the cover glass was pried off. The preparations were washed in PBS to remove traces of acetic acid and then treated with 0.1% glycine in PBS. The nonspecific binding sites were blocked by incubation of the preparations in 5% milk powder (Semper, Sweden) in PBS for 30 min. The PBS-rinsed preparations were double-stained, first with 50 μl biotinylated antibody (conc. 10–15 μg/ml) for about 45 min and then with 40 μl of FITC-conjugated second antibody (conc. 15–20 μg/ml) mixed together with 30 μl Texas Red-Streptavidin (conc. 1 μg/ml) for 45 min. Finally, the preparations were rinsed in PBS, then in distilled water, and mounted in DAKO fluorescence mounting medium.

Labeling of transcription sites by incorporation of BrUTP

Salivary glands were isolated from fourth instar larvae and incubated in hemolymph containing 4 mM of BrUTP (Sigma-Aldrich, Sweden) for 30 min in a humid chamber. After incubation the glands were washed in ZO culture medium and processed for polytene chromosome preparation and immunostaining as described above.

DRB treatment

Isolated salivary glands were incubated for 60 min in hemolymph containing 20 μg/ml of 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole (DRB) (Sigma-Aldrich), a chemical agent known to inhibit RNA Pol II transcription.

RNase treatment

After removal of the coverslips and rinsing in PBS, the chromosome preparations were treated with RNase A (200 μg/ml) for 30 min at room temperature and stained with various antibodies for immunofluorescence analysis.

Immunofluorescence analysis

Double-stained preparations were examined under epifluorescence using filters suitable for the indicated fluorochromes and photographed on Kodak Tmax 400 film. The preparations were also examined and photographed in a Zeiss Axiophot fluorescence microscope equipped with a cooled CCD camera (Photometrics Nu 200/CH250). The signals were analyzed on a Macintosh Quadra 950 computer using Smart-capture software (Digital Scientific, UK).

The intensity of each hrp45-labeled locus was depicted in a relative scale ranging from 1 to 5 where 1 represents weakly labeled loci and 5 represents the loci most strongly labeled.

Results

Specificity of mAbs used in the present study

In the present study we have used mAbs 2E4 and 4F9 raised against SR protein hrp45 and an hnRNP protein

hrp36 of *C. tentans*, respectively (Wurtz et al. 1996). To analyze the distribution of snRNP proteins, we used mAbs 4G3 against the U2B″ protein and Y12 against the Sm epitope of snRNP proteins. The specificity of these antibodies against their respective proteins in nuclear extract of *C. tentans* tissue culture cells was analyzed by Western blot (Fig. 1). As shown in Fig. 1, the antibodies against hrp36, hrp45, and U2B″ recognized the corresponding proteins with high specificity. The anti-Sm antibody bound to a small set of putative Sm proteins (cf. Kiseleva et al. 1994).

The hnRNP protein hrp36 and snRNPs associate with most transcriptionally active loci on polytene chromosomes

The nuclei of larval salivary gland cells in *C. tentans* possess four polytene chromosomes designated as chromosome I, II, III, and IV. Each chromosome contains many

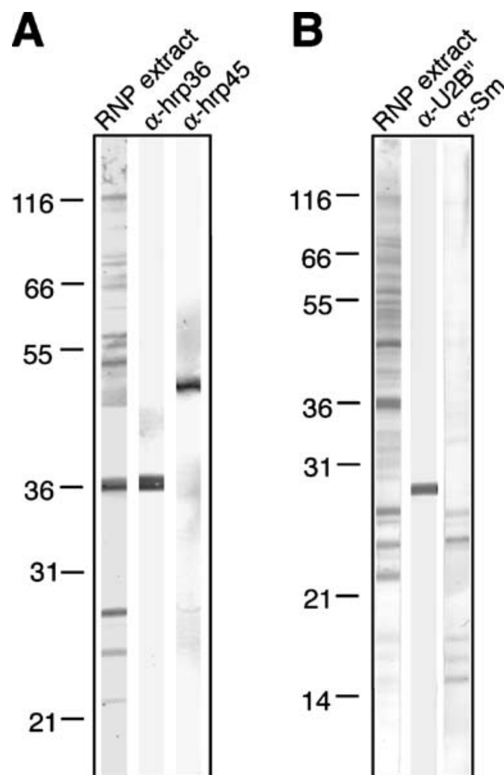

Fig. 1 Immunoblot detection of hnRNP protein hrp36, SR protein hrp45, and snRNP proteins in a *C. tentans* nuclear extract from tissue culture cells. The proteins of the nuclear extract were separated by SDS polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose membrane, and probed with monoclonal antibodies. In subpanel **a**, *lane 1* shows the Coomassie-stained nuclear extract and *lanes 2* and *3* show the Western blot using mAb 4F9 against hrp36 and mAb 2E4 against hrp45. In subpanel **b**, *lane 1* shows the nuclear extract stained with Coomassie and *lanes 2* and *3* show Western blot using mAb Y12 against the Sm epitope and the 4G3 against U2B″ epitope of snRNP proteins. The positions of the molecular size markers (kilodaltons) are presented to the *left* of the Coomassie-stained *lanes*

transcriptionally active loci. The highly active sites and/or the sites where larger transcripts are synthesized appear as swollen structures, the puffs, which contain nascent RNP complexes (Daneholt 2001). Thus, different puffs contain different types of nascent RNP complexes, which can be analyzed by antibody staining at the light microscopy level. Chromosome IV contains three exceptionally large puffs, referred to as the BRs (BR1, BR2, and BR3). These puffs are the loci of highly active sp genes and contain large amounts of nascent mRNP complexes. In addition to the BRs, several other smaller puffs were mapped as loci for sp genes (Wieslander 1994).

To visualize transcriptionally active loci on polytene chromosomes by immunofluorescence, we incorporated BrUTP, which was subsequently detected by FITC-conjugated anti-BrdU antibody. We found approximately 135 fluorescent bands representing transcriptionally active loci in the four polytene chromosomes of *C. tentans*. In the double-labeling experiment when the same chromosomes were analyzed for the location of hrp36, we found that the distribution of hrp36 and that of the most transcriptionally active loci visualized by BrUTP coincide. Immunofluorescence analysis of chromosome II and IV double-labeled with hrp36 and BrUTP is presented in Fig. 2. Almost identical fluorescent banding pattern can be seen in the two panels of the figure. Approximately 130 loci were labeled with hrp36. A small number of loci (about five) were labeled with BrUTP but not with hrp36. It could be that the anti-hrp36 epitope is not exposed at these loci or that these loci are not sites for RNA polymerase II transcription.

Further, we analyzed the distribution of snRNP proteins in relation to the transcriptional activity (Fig. 3). In double-labeling experiments, we observed that most of the BrUTP-labeled loci also harbored snRNP proteins detected by the anti-snRNP antibodies 4G3 and Y12 (Fig. 3). Most of the loci showed a moderate snRNP protein labeling. However, a few loci showed a relatively high intensity of snRNP labeling while some exhibited a relatively low intensity. Nevertheless, we conclude that the overall distribution of snRNP proteins also largely coincided with the BrUTP labeling. The distributions of BrUTP and snRNP protein on chromosome I and IV are displayed in Fig. 3. Two loci showing snRNP labeling of higher intensity are indicated with arrows in Fig. 3. The locus indicated with arrow on chromosome IV is BR3, which synthesizes a pre-mRNA containing 38 introns (Paulsson et al. 1990). The same distribution of snRNP proteins was observed when snRNP proteins were compared with hrp36 (data not shown).

To confirm that hrp36, snRNPs, and BrUTP are associated with RNA, chromosomes were treated with RNase A before immunolabeling. After RNase A treatment, hrp36, snRNPs, and BrUTP labeling could not be detected by immunofluorescence analysis (data not shown). Further,

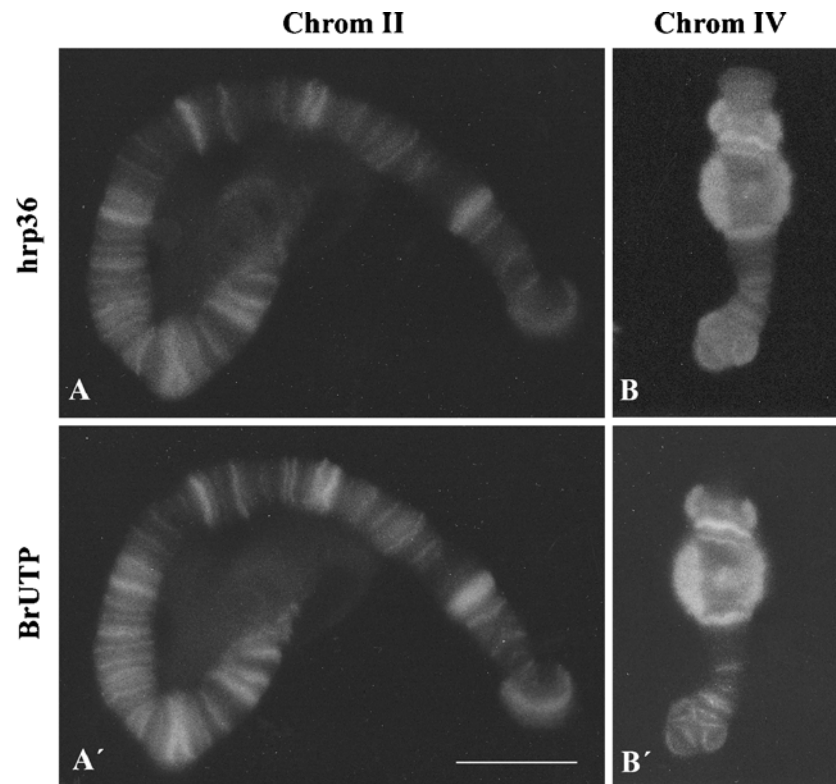

Fig. 2 Distribution of hrp36 and transcriptional activity (BrUTP labeling) along polytene chromosomes II and IV of *C. tentans* as examined by immunofluorescence microscopy. Squash preparations of salivary glands incubated in hemolymph containing BrUTP were challenged with FITC-conjugated anti-BrdU antibody and biotinylated mAb 4F9. The labeling of 4F9 was visualized by Texas Red-Streptavidin. *A* and *A′* Chromosome II; *B* and *B′* chromosome IV. *Bar*=10 μm

when salivary glands were treated with DRB, an inhibitor of RNA polymerase II transcription, we found a drastically reduced immunofluorescence signal (data not shown). The negative control experiments with either anti-von Willebrand factor VIII antibody or with only secondary antibody showed no significant labeling.

Thus, we infer that hrp36 and snRNP proteins bind to most primary transcripts as their labeling largely overlaps the distribution pattern of BrUTP. Further, it is also indicated that loci generating transcripts with a large number of introns show higher intensity of snRNP labeling as found in the case of BR3.

SR protein hrp45 binds preferentially with nascent transcripts at a small number of loci

To reveal the association of SR protein hrp45 with different nascent transcripts, we studied the distribution of hrp45 on transcriptionally active loci in relation to the distribution of hrp36 and snRNP proteins. The hrp45 and hrp36 proteins were also simultaneously analyzed in double-labeling experiments to eliminate the possibility of preparative and/or physiological variations.

Polytene chromosomes double-stained with either hrp45 and BrUTP or hrp45 and hrp36 revealed that the distribution of hrp45 did not follow the labeling patterns of BrUTP and hrp36 (Fig. 4). Rather, hrp45 showed higher intensity of labeling at a small number of loci. In total, we detected about 20 loci (depicted with relative labeling intensity from 2 to 5) preferentially associated with hrp45. Of these, 11 were strongly labeled loci (depicted by labeling intensity 3 and more in Table 1), which included the three BRs, a small puff on chromosome IV, and seven other smaller puffs on chromosomes I, II, and III (Table 1). Although hrp45 was also found distributed on many other loci, the relative intensity of labeling of these loci was low compared to the 20 loci mentioned above. All such weakly labeled loci displayed a labeling intensity below 2.

The distributions of hrp45 and hrp36 were analyzed on all four chromosomes, and data on loci preferentially associated with hrp45 were collected. Images of double-labeled chromosome I, II, and IV, which harbor sp genes, are presented in subpanels a, b, and c of Fig. 5, respectively. The hrp45 antibody revealed only a few strongly labeled bands, while a large number of loci are labeled with the hrp36 and snRNP antibodies. To ascertain that the

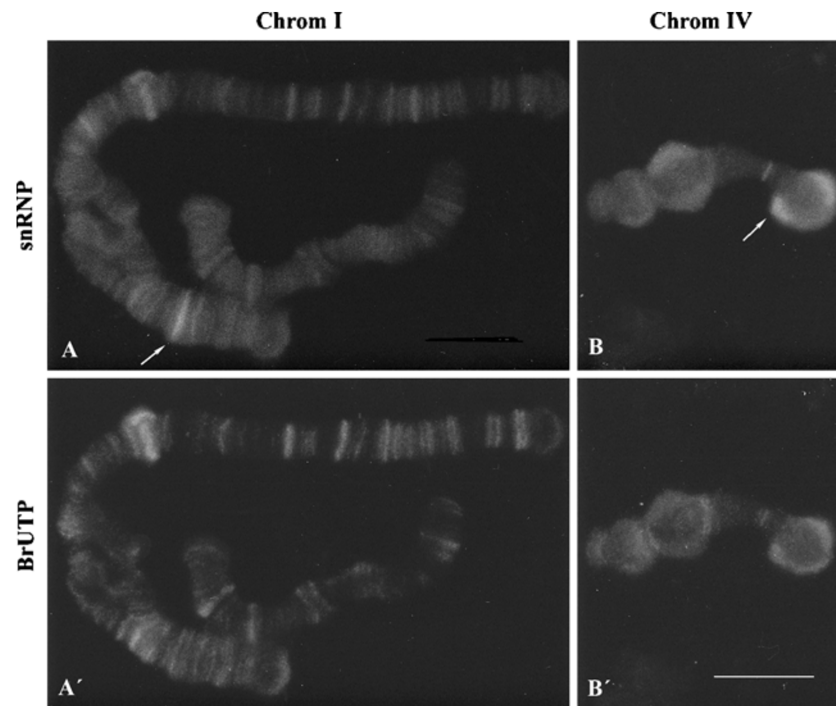

Fig. 3 Distribution of snRNP proteins and transcriptional activity (BrUTP labeling) along polytene chromosomes I and IV of *C. tentans* as studied by immunofluorescence microscopy. Squash preparations of BrUTP-incorporated salivary glands were incubated with biotinylated mAb Y12 and FITC-conjugated anti-BrdU antibody. The labeling of Y12 was visualized by Texas Red-Streptavidin. *A* and *A′* Chromosome I; *B* and *B′* chromosome IV. Loci showing higher intensity of snRNP labeling are indicated by *arrows*. *Bar*=10 μm

differential distribution of hrp45 is a constant feature, we analyzed its distribution in double-labeling experiments using different fluorochromes in various combinations and found that the observed labeling pattern was not altered with changes of the fluorochrome (data not shown). It is also not due to the differential gene activity as smaller puffs labeled with hrp45 do not show any significantly higher intensity with BrUTP labeling. We therefore conclude that the SR protein hrp45 preferentially binds to a small number of loci that include the three BRs and a few smaller puffs.

The hrp45-enriched loci are the transcription sites of sp genes

To identify the transcripts with which hrp45 binds preferentially, we compared the hrp45-labeled loci with the location of known genes mapped on polytene chromosomes of *C. tentans* (Wieslander 1994). It is interesting to note that we found that 8 out of the 11 loci, which showed labeling intensity higher than 3, harbor genes coding for sp. The eight hrp45 intensely labeled loci that generate sp gene transcripts are I-5C, I-17A, I-17B, II-5C, IV-2A, IV-3B, IV-5B, and IV-6A/B. The hrp45 preferentially associated loci and corresponding sp genes are summarized in Table 1. Because sp genes are active on polytene chromosomes during the larval stage and form puffs containing nascent pre-mRNPs and because the hrp45 labeling of these puffs is RNase A-sensitive, we conclude that hrp45 preferentially binds to sp gene transcripts.

Discussion

The hrp36 and snRNP proteins were found associated with almost all transcriptionally active loci in *C. tentans* polytene chromosomes. The labeling intensity of these proteins broadly followed the transcription levels of the loci baring a few exceptions. Earlier studies on simultaneous detection of hnRNP and snRNP proteins on polytene chromosomes of *Drosophila* by immunofluorescence also revealed that most transcripts associate with both types of proteins (Matunis et al. 1993). However, hrp45 was found preferentially distributed in a smaller number of loci, which showed exceptionally high intensity of hrp45 labeling. In total, 20 loci with prominent hrp45 labeling were observed, and they were recorded on all four polytene chromosomes. Of these, 9 loci are depicted with labeling intensity 2, whereas 11 loci had a labeling intensity of 3 or more (Table 1).

Intense labeling of a splicing factor in a gene locus could reflect the presence of many introns and intense splicing. Such a situation was seen in the present study for the BR3 locus in the case of staining with the anti-snRNP protein antibodies. The BR3 transcripts contain 38 introns

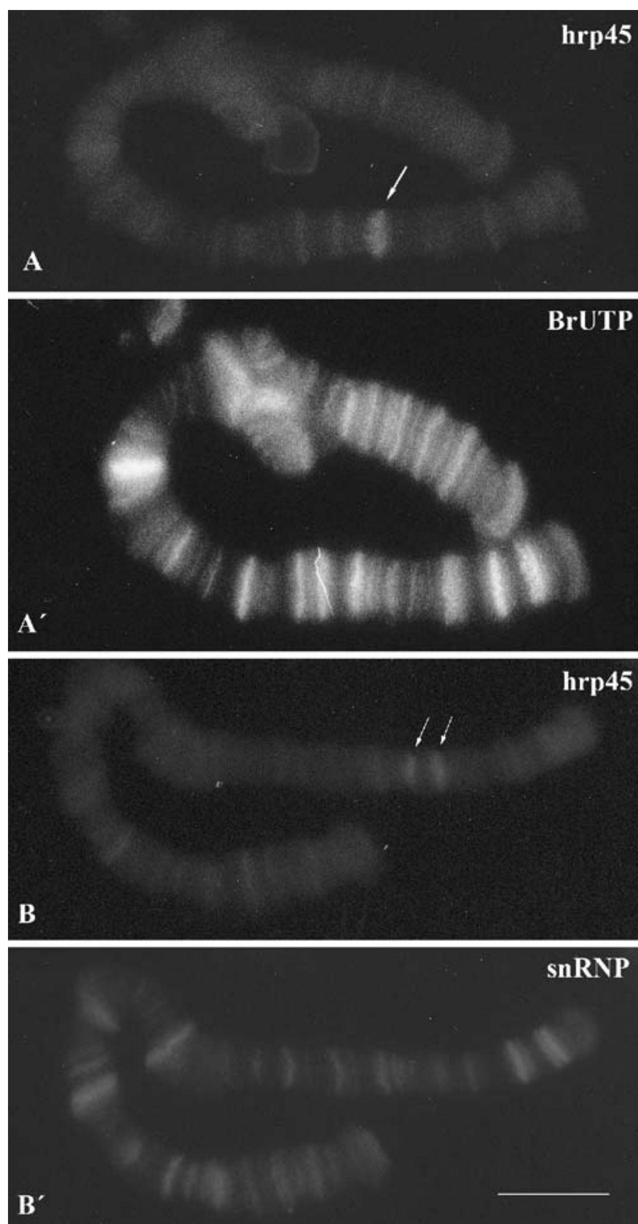

Fig. 4 Immunofluorescence visualization of hrp45 in relation to transcriptionally active loci (labeled by BrUTP) and to snRNP proteins on polytene chromosome I of *C. tentans*. For visualization of hrp45 in relation to transcriptional activity, squash preparations of BrUTP-incorporated salivary glands were incubated with biotinylated mAb 2E4 and FITC-conjugated anti-BrdU antibody. The labeling of 2E4 was visualized by Texas Red-Streptavidin. However, for visualization of hrp45 in relation to the distribution of snRNP protein, squash preparations of salivary glands were incubated with biotinylated mAb Y12 and FITC-conjugated mAb 2E4 antibodies. Labeling of Y12 was visualized by Texas Red-Streptavidin. **a***A′* Chromosome I labeled with hrp45 and BrUTP. **b***B′* Chromosome I labeled with hrp45 and snRNP. Loci showing preferential association with hrp45 are indicated by *arrows*. *Bar*=10 μm

(Paulsson et al. 1990) and are largely cotranscriptionally spliced (Wetterberg et al. 2001). Such a correlation between intensity of labeling has earlier been seen in the BR3 locus after staining with anti-snRNP proteins (Kiseleva et al. 1994; Björk et al. 2006). In the case of staining for hrp45, we did not find any correlation between staining intensity and number of introns/splicing activity. The BR1 and BR2 genes contain four introns and the BR3 gene 38 introns. However, no clear difference could be detected in staining intensity in these loci. This is consistent with the previous findings that hrp45 binds the exceptionally long exons of the BR1 and BR2 transcripts (Alzhanova-Ericsson et al. 1996).

Intense labeling of hrp45 could also reflect a higher level of transcription and hence more nascent transcripts. However, when hrp45 labeling is analyzed in relation to BrUTP incorporation, we did not find significantly higher BrUTP incorporation in hrp45 intensely labeled loci.

On further analysis, it was found that 8 of the 11 strongly labeled loci are the sites of sp genes. The hrp45 labeling being sensitive to RNase A treatment confirms that hrp45 binds to the sp pre-mRNA synthesized in these loci. The eight strongly labeled loci include the three BRs, which are well-studied sp gene loci on chromosome IV. It was shown that the BR1 and BR2 harbor actively transcribing genes of 35–40 kb of nucleotides, which code for large-sized sp

Table 1 Loci strongly labeled with anti-hrp45 antibody 2E4 on polytene chromosomes of *C. tentans* and corresponding known locations of secretary protein genes

Chromosome	Polytene chromosome map location of loci	Relative intensity of labeling	Known secretary protein genes on hrp45-labeled loci
I	3B	2	
	5C	4	sp17
	7A	2	
	11A	2	
	17A	4	sp240/420
	17B	4	sp115, sp140
II	5C	3	sp38-40A, sp38-40B
	6B	3	
	13B	2	
	13C	2	
III	1C	2	
	2C	2	
	12C	3	
	14A	3	
IV	2A(BR1)	5	spIa, sp195
	3B(BR2)	5	spIb, spId
	5B	3	sp12
	5C	2	
	6A/B(BR3)	4	sp185
	6C	2	

The relative intensity of labeling of loci is depicted in a scale from 1 to 5. Labeling intensity 1 represents weakly stained loci (not included in this table) whereas 5 represents the most strongly labeled ones. The loci showing labeling intensity 2 or more are listed in this table

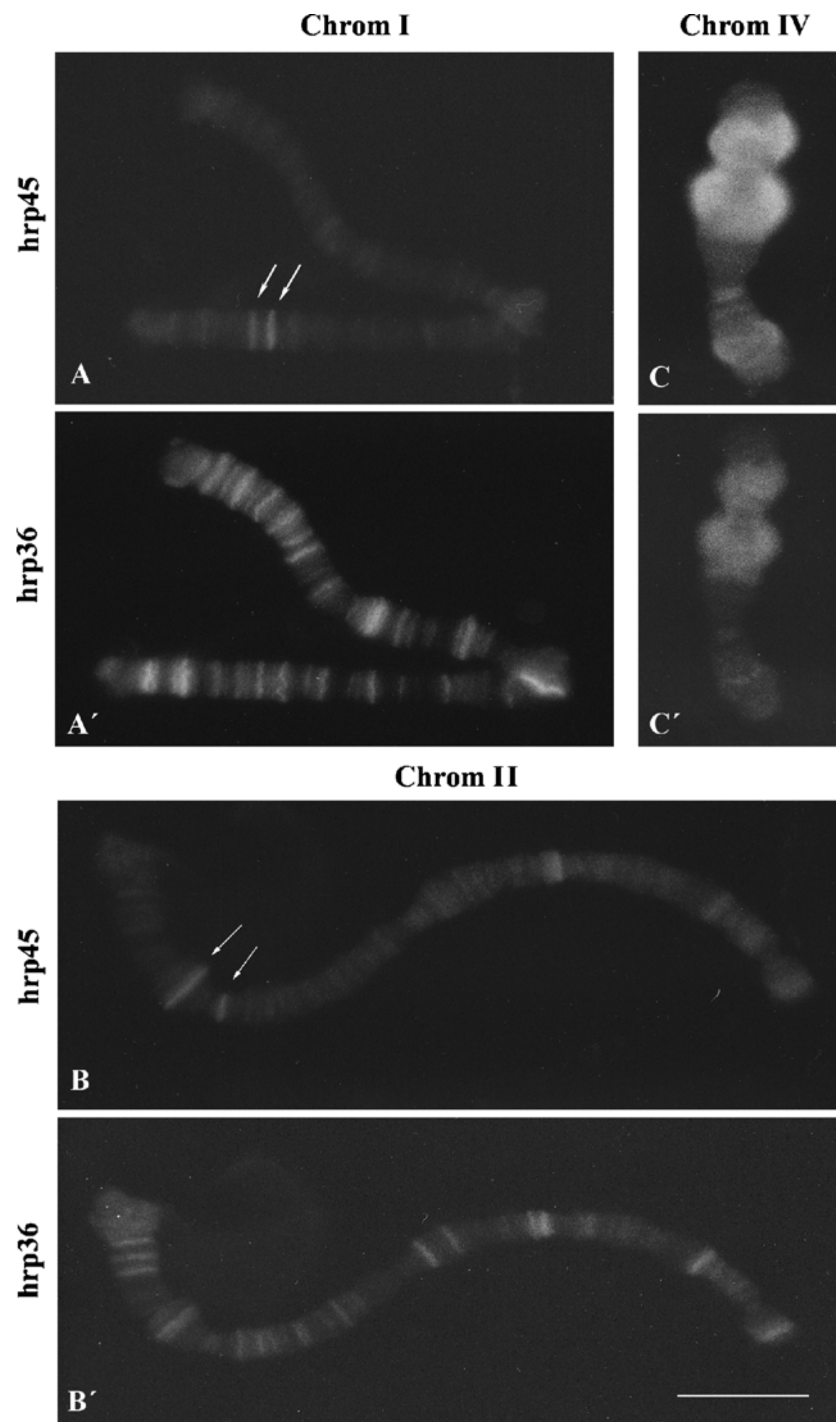

Fig. 5 Comparison of the distributions of hrp45 and hrp36 on polytene chromosomes I, II, and IV of *C. tentans* using immunofluorescence microscopy. Squash preparations of salivary glands were incubated with biotinylated mAb 2E4 and FITC-conjugated mAb 4F9. The labeling of 2E4 was visualized by Texas Red-Streptavidin. **a***A′* Chromosome I. **b***B′* Chromosome II. **c***C′* Chromosome IV. The loci showing preferential association with hrp45 are indicated by *arrows*. *Bar*=10 μm

proteins (about 1 MDa) that form the proteinaceous larval tube (Case and Wieslander 1992).

The fact that hrp45 preferentially associates with sp gene transcripts in salivary gland cells of *C. tentans* raises questions about their possible roles in the regulation of the activity of sp genes. The hrp45 protein exhibits high sequence similarity to *Drosophila* SRp55/B52 (Champlin et al. 1991; Roth et al. 1991). It was reported

that SRp55/B52 not only functions as a splicing factor but also performs an important role in embryonic development (Ring and Lis 1994). Immunoelectron microscopic analysis showed that hrp45 is added continuously to the growing BR RNP complex concomitant with transcription and is bound essentially along the entire BR transcript. Remarkably, it was found to be associated along the entire exon sequence rather than to be confined to specific sites close to introns (Alzhanova-Ericsson et al. 1996). In a recent study, Björk et al. (2006) reported that hrp45 is recruited onto growing pre-mRNAs together with the Ct-RSF, previously described as hrp23 (Sun et al. 1998). The hrp45 protein was also found to be associated with nucleoplasmic BR particles and the particles translocating through the nuclear pores (Alzhanova-Ericsson et al. 1996).

The hrp45 protein could act as a splicing factor. This is supported by the observation that hrp45 activates splicing of Fushi tarazu transcripts in S100 extract from *C. tentans* cells (Björk et al. 2006). However, the facts that at the same time hrp45 binds essentially along the entire BR transcript and accompanies the BR RNA to the nuclear pore indicate additional functions in regulation of gene expression. The observation in our study that hrp45 preferentially associates with sp gene transcripts also indicates that this SR protein performs some specific functions in the biogenesis of these particular transcripts. It is most close at hand to suggest that hrp45 is somehow required for speedy and efficient processing and transport of the sp gene transcripts in salivary gland cells due to the exceptional need for the encoded proteins during the larval stage of development. It was shown that the SR protein 9G8 serves as an adapter protein for the TAP/NXF1 receptor that mediates essential interactions with nuclear pore complex (NPC) in the process of nucleocytoplasmic transport and facilitates export of mRNA (Huang et al. 2003). Because hrp45 remains associated with BR particles during translocation through the NPC, it is possible that hrp45 might be acting as an export adaptor for the sp gene transcripts. Further study is required to ascertain the specific role of hrp45 and also to understand its mechanism of preferential binding with the sp gene transcripts.

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