Distribution of B52 Within a Chromosomal Locus Depends on the Level of Transcription

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Drosophila B52 protein is a homologue of human ASF/SF2 that functions in vitro as an essential pre-mRNA splicing factor. Immunofluorescence analysis of polytene chromosomes has shown that B52 generally colocalizes with RNA polymerase II; however, in contrast to other splicing factors, B52 brackets RNA polymerase II at highly active heat-shock puffs. Also, UV cross-linking in nonpolytene cells has shown that B52 cross-links in vivo to DNA flanking the highly active transcription units. Here, we find that the distribution of cross-linked B52 at heat-shock loci depends on transcription levels. Heat shocks at low and moderate temperatures, which induce corresponding levels of transcription, recruit B52 both to transcribed DNA and to flanking DNA, whereas a full heat-shock induction concentrates B52 on the DNA that brackets the entire activated region. We have also identified a 46-kDa protein from *Chironomus tentans* that binds *Drosophila* B52 antibodies and has a distribution on chromosomes analogous to B52. This protein is found throughout the moderately transcribed Balbiani rings. However, when transcription at these rings is hyperinduced to levels comparable to fully induced *Drosophila* heat-shock genes, the protein is restricted to the boundaries of highly decondensed chromatin. We suggest that B52 tracks to chromatin fibers that are folding or unfolding, and we discuss this in light of B52's proposed roles in pre-mRNA splicing and control.

INTRODUCTION

Drosophila B52 is a homologue of the human protein ASF/SF2 (Champlin *et al.*, 1991). ASF/SF2 confers splicing activity to splicing-inactive S-100 extracts, and the concentration of ASF/SF2 also influences the utilization of alternative splice sites in vitro (Ge *et al.*, 1991; Krainer *et al.*, 1991; Fu *et al.*, 1992). B52 can substitute for both of these activities of ASF/SF2 in in vitro mammalian splicing reactions (Mayeda *et al.*, 1992). If B52 functions as a splicing factor in vivo in a manner like that seen in vitro, then it is not surprising that B52 and RNA polymerase II are found, in general, to colocalize on polytene chromosomes (Champlin *et al.*, 1991). Both proteins are expected to be in close association with newly made transcripts. However, it is more difficult to reconcile B52's proposed role as a splicing factor with the finding that B52 is recruited to the borders of highly active heat-shock puffs and cross-links in vivo to DNA that flanks the transcribed regions of the fully activated

heat-shock genes (Champlin *et al.*, 1991). One explanation is that B52 interacts with both a specific chromatin structure and RNA. For example, B52 may target to a feature of transcriptionally active chromatin, thereby positioning it for splicing or for influencing splice site choice. The apparent mistargeting of B52 to the borders of the RNA polymerase II distribution at highly active heat-shock loci, which contain genes with no introns, may reflect a targeting of B52 to chromatin undergoing decondensation at the borders of highly active transcription units.

Fully induced *Drosophila* heat-shock genes have a very high density of RNA polymerase II, estimated to be about one polymerase per 80 basepairs (bp) of DNA (Gilmour and Lis, 1986; O'Brien and Lis, 1991). Histones remain on the transcribed regions of genes expressed at this exceptionally high level (Solomon *et al.*, 1988; Ericsson *et al.*, 1990), but normal nucleosome organization is disrupted (Wu *et al.*, 1979; Bjorkroth *et al.*, 1988; Nacheva *et al.*, 1989). Electron microscopic examination of the chromatin fiber of hyperinduced Balbiani ring genes, which are also transcribed at this high level, reveal that the transcribed regions are in an ex-

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tended 5-nm chromatin fiber devoid of the 10-nm nucleosomal fiber or beads (Bjorkroth *et al.*, 1988). At lower levels of transcription, the distances between adjacent polymerases on the Balbiani ring genes increase, allowing the 10-nm and even 30-nm fibers to refold between the adjacent RNA polymerase II complexes. Therefore, an obvious structural difference exists between the majority of genes and the most highly transcribed genes. For most genes, a transition from condensed to decondensed chromatin occurs with the passage of each RNA polymerase, that is, throughout the body of the gene; whereas for genes transcribed at exceptionally high levels, this transition in structure occurs only at the borders of the transcribed regions.

Here, we determine how the distribution of B52 within a locus varies with changes in the density of RNA polymerase II. This distribution is examined in *Drosophila* cells at high resolution in vivo using a UV-cross-linking method (Gilmour *et al.*, 1991). Furthermore, because we find that both monoclonal (mAb) and polyclonal antibodies to *Drosophila* B52 cross-react with a single 46-kDa protein from *Chironomus tentans*, we examine the distribution of this cross-reactive protein cytologically on the developmentally regulated Balbiani rings under normal conditions and where transcription of genes in the rings is hyperinduced. We compare this distribution to that of B52 on heat-shock puffs. Finally, we discuss the possible targets recognized by these proteins and suggest a role of this target recognition in RNA splicing.

MATERIALS AND METHODS

Culturing of *Chironomus*

Staged fourth instar *Chironomus tentans* were gracious gifts from Bob Bode, New York State, Albany; Peter Nolan, Massachusetts Environmental Protection Agency, Boston; and Steven Case, University of Mississippi, Jackson. Animals were maintained at 18°C in aerated cultivation water (Mahr *et al.*, 1980). Pilocarpine treatment was carried out by adding pilocarpine nitrate (P6628, Sigma, St. Louis, MO) directly to the cultivation water (0.1 mg/ml) and maintaining the animals in an aerated chamber at 18°C (Mahr *et al.*, 1980).

UV Cross-linking

UV cross-linking was done as described (Champlin *et al.*, 1991; Gilmour *et al.*, 1991) on *Drosophila* Schneider line 2 (SL2) cell cultures with minor modifications. Briefly, SL2 cells growing in jacketed spinner flasks were submitted to a 20 min heat shock at 36.5°C. Cells were then chilled on ice and UV irradiated while still in culture media. DNA and protein-DNA adducts were copurified on a CsCl gradient containing 0.5% Sarkosyl (Sigma, St. Louis, MO). After dialysis, this material was digested with restriction enzymes, and B52-DNA adducts were immunoprecipitated by sequential incubations with 6 µl of B52 mAb from mouse ascites fluid and 350 µl of sheep anti-mouse IgG conjugated to Dynabeads M-450 magnetic beads (Dyna, Oslo, Norway) per sample. Immunoprecipitates were treated with proteinase K, and the DNA was characterized by Southern blot analysis. Nonheat-shock samples were processed identically except that the heat shock was omitted.

Western Blotting

Five fourth instar *Chironomus* larval salivary glands were ground with a microcentrifuge mortar and pestle (Kontes, Vineland, NJ) in 100 µl of 2× sodium dodecyl sulfate (SDS) loading buffer (Schleif and Wensink, 1981 #437). Proteins were separated by electrophoresis on 8% SDS-polyacrylamide gels and electrophoretically transferred to Immobilon-NC (Millipore, Bedford, MA) (Towbin *et al.*, 1979). Protein was detected by sequential incubations of the blots with a 1:1000 dilution of mAb or affinity-purified polyclonal antibody to *Drosophila* B52 (Champlin *et al.*, 1991), biotinylated goat anti-mouse or anti-rabbit IgG (Vector, Burlingame, CA), ABC-alkaline phosphatase (Vector), and Western Blue (Promega, Madison, WI). Tween 20 (Sigma) at 0.05% was added to incubations and washes when the mAb was used.

Histological Procedures and Microscopy

Methods for the dissection of *Chironomus* salivary glands, fixation, and squashing were the same as those used for *Drosophila* chromosomes in Champlin *et al.* (1991). Briefly, glands were dissected in Buffer A with 1% Triton X-100. Glands were fixed for 30 s in this solution plus 3.7% paraformaldehyde. Squashes were then prepared in 50% glacial acetic acid/3.7% paraformaldehyde. Following freezing in liquid N₂ and dehydration in 95% ethanol, slides were rehydrated in TBS (10 mM tris(hydroxymethyl)aminomethane-HCl pH 7.4, 150 mM NaCl) and incubated sequentially under coverslips in a humid chamber with the following: TBS/10% normal goat serum (NGS) for 1 h, TBS/10% NGS with 1:200 B52 mAb and 1:200 affinity-purified B52 polyclonal antibody or 1:50 affinity-purified RNA polymerase II antibody for 2 h (Champlin *et al.*, 1991), and TBS/10% NGS with 1:200 fluorescein isothiocyanate-conjugated goat anti-mouse and 1:200 rhodamine-conjugated goat anti-rabbit secondary antibodies (Cappel, Cochranville, PA). Photography was done using a Zeiss Universal fluorescence microscope (Thornwood, NY) and M63 camera with Kodak TMZ-P3200 film (Rochester, NY).

RESULTS

The Distribution of B52 on *Drosophila* Heat-Shock Loci Is Related to the Level of Transcription

The distributions of B52 and RNA polymerase II are similar on polytene chromosomes as viewed by fluorescence microscopy, except at the large heat-shock puffs where B52 brackets RNA polymerase II (Champlin *et al.*, 1991). This separation of B52 and RNA polymerase II could be a consequence of the added resolution provided by the large puffs; however, at least some large developmental puffs of *Drosophila* do not show a clear bracketing pattern (unpublished data). As an alternative explanation, we consider that the separation of polymerase and B52 may be a consequence of the high density of transcription complexes on heat-shock genes, a density higher than that of many developmental puffs. This high density of RNA polymerase complexes leaves chromatin in a highly extended configuration (as described in the INTRODUCTION), and this may alter the targeting of B52.

The densities of RNA polymerase II on heat-shock genes can be varied systematically by exposing cells to different temperatures. Exposure of SL2 cells to 30, 33, and 36.5°C results in an average of one RNA polymerase II molecule every 4000, 266, and 80 bp of DNA,

Figure 1. Distribution of B52 at the 67B locus at various heat shock temperatures. The in vivo concentration of B52 on the small *Sal* I fragment flanking the heat shock genes was compared to that on the large *Sal* I fragment that contains the *hsp27* and *hsp23* genes using the UV cross-linking method. Intact cells were irradiated as described in MATERIALS AND METHODS, and specific protein-DNA adducts were immunoprecipitated with B52 antibody. The coprecipitated DNA (B52 impt.) was quantified on the Southern blots shown by comparing these signals to that from fractions of the total sample (0.2 and 0.02%) processed in parallel for each sample. Nonheat-shock cells (23°C) and fully heat-shocked cells (36.5°C) are shown in the top panels. Intermediate heat shocks are shown in the bottom panels (31.5 and 33°C). The restriction map summarizes the distribution of B52 in fully heat-shocked cells seen previously (Champlin *et al.*, 1991), with black bars indicating DNA fragments that were found to cross-link with B52 during a 36.5°C (full) heat shock. The restriction fragments examined are aligned in each case to the corresponding crosslinking result. Restriction sites used: B, *Bam*HI; H, *Hind*III; and S, *Sal* I. Southern blot filters were probed with corresponding subcloned DNA fragments from λ Dm202 (Costlow and Lis, 1984).

respectively (O'Brien and Lis, 1991). We examined the distribution of B52 at the 67B locus at high resolution by UV cross-linking in SL2 cells that had been heat shocked at a variety of temperatures. In the absence of heat shock, we detected little or no B52 association with the DNA at this locus (Figure 1), as was seen previously (Champlin *et al.*, 1991). Increasing temperatures of heat shock increase the level of transcription of the heat shock genes and increased the amount of B52 associated with a restriction fragment flanking the *hsp27* gene. In contrast, the amount of B52 associated with a fragment spanning the *hsp23* and *hsp27* genes first increased and then decreased as the transcription of heat-shock genes was increased (Figure 1). In this experiment, a small amount of this fragment was detected in the B52-immunoprecipitated 36.5°C sample, which in earlier cross-linking experiments was not detected (Champlin *et al.*, 1991).

This is perhaps due to the increased signal to noise provided by using magnetic beads in the immunoprecipitations described here (see MATERIALS AND METHODS) or to small variations in the heat-shock response between experiments.

We also examined the distribution of B52 at the 87A heat-shock locus at different heat-shock temperatures. Increasing temperatures of heat shock again led to increased association of B52 with a restriction fragment that includes the 3' half of the *hsp70* genes (Figure 2). At 36.5°C, full heat shock, no detectable B52 associated with the 5' half of *hsp70*, consistent with earlier cross-linking experiments (Champlin *et al.*, 1991). However, at both 31.5 and 33°C, significant amounts of B52 cross-linked to this fragment. Thus, B52 associated with transcribed regions of heat shock loci when transcription is induced to low or moderate levels, but it concentrates on the regions bordering the transcribed regions when transcription levels are extremely high.

Figure 2. Distribution of B52 at the 87A locus at various heat-shock temperatures. In vivo concentration of B52 on *Sal* I-*Bam* I fragment that includes the 3' end of the *hsp70* gene was compared to that on the 5' *Sal* I fragment by UV cross-linking as described in Figure 1. Nonheat-shock cells (23°C) and fully heat-shocked cells (36.5°C) are shown in the top panels. Intermediate heat shocks are shown in the bottom panels (31.5 and 33°C). The restriction map summarizes the distribution of B52 in fully heat-shocked cells seen previously (Champlin *et al.*, 1991) with black bars indicating DNA fragments that were found to cross-link to B52 during a full heat shock (36.5°C), and open bars indicating fragments that do not cross-link efficiently with B52 during heat shock. The restriction fragments examined are aligned in each case to the corresponding cross-linking result. Restriction sites used: B, *Bam*HI; R, *Eco*RI; and S, *Sal* I. Southern blot filters were probed with the corresponding cloned DNA fragments from the 87A locus.

B52 Antibodies Cross-React with a *Chironomus* Protein Localized to Transcriptionally Active Chromatin

The midge, *Chironomus tentans*, is a Dipteran separated from *Drosophila melanogaster* by 65–135 million years of evolution (Beverley and Wilson, 1984). Both mAbs and polyclonal *Drosophila* B52 antibodies cross-react strongly with a 46-kDa protein from *Chironomus* fourth instar larval salivary glands (Figure 3).

Polytene chromosomes from fourth instar larval salivary glands were prepared for indirect immunofluorescence. The mAbs and polyclonal *Drosophila* B52 antibodies were used for double-labeling, with both antibodies localized to many interbands and puffs including the large Balbiani rings (Figure 4). This double-labeling experiment demonstrates that the staining patterns for the two antibodies are identical. Furthermore, the distribution is very similar to that reported for RNA polymerase II (Sass, 1982). When chromosomes were double-labeled with antibodies to *Drosophila* B52 and RNA polymerase II, the staining patterns of the two antibodies appeared to be quite similar (Figure 4). B52 and RNA polymerase II antibodies labeled the same sites including the heavily labeled and most transcriptionally active sites, Balbiani rings 1 and 2.

Distribution of B52 Antigen on Pilocarpine-stimulated Balbiani Rings Resolves from that of RNA Polymerase II

The drug pilocarpine causes a rapid increase in the expression of Balbiani ring genes. Three effects of the drug on *Chironomus* have been described: uncoordinated movement, altered morphology of the salivary gland associated with expulsion of the contents of the lumen and an increase in the sizes of Balbiani rings 1 and 2 (Mahr *et al.*, 1980). The Balbiani ring genes encode proteins secreted into the lumen of the salivary gland. It is thought that drug-induced contractions of the animal cause an expulsion of the contents of the lumen, thereby indirectly triggering an increase in transcription of the genes (Mahr *et al.*, 1980). The increased transcription caused by pilocarpine results in an extension of the chromatin axis that increases the size of the Balbiani rings (Widmer *et al.*, 1984; Olins *et al.*, 1986). The RNA polymerase II densities on pilocarpine-stimulated Balbiani ring genes have been estimated to be about one molecule per 80 bp (Widmer *et al.*, 1984), and these genes are completely devoid of 10- and 30-nm fibers (Bjorkroth *et al.*, 1988; Ericsson *et al.*, 1990). In the absence of pilocarpine, the developmentally expressed Balbiani ring genes have an average of one polymerase II molecule per 300 bp with a variability in spacing that is frequently sufficient to allow refolding of the 10- and 30-nm fibers between adjacent polymerase

Figure 3. *Chironomus* proteins cross-reactive with *Drosophila* B52 antibodies. Total proteins from *Chironomus tentans* fourth instar larvae were analyzed by Western blot using the *Drosophila* B52 monoclonal (mono.) and polyclonal (poly.) antibodies. Five larval salivary glands were ground with a microcentrifuge mortar and pestle in 100 µl of 2× SDS loading buffer. The lanes contained 1 µl and 10 µl of this protein extract.

molecules (Bjorkroth *et al.*, 1988). Thus, the chromatin structures of normal and hyperinduced Balbiani ring genes differ dramatically in whether this unfolding and refolding is occurring on the transcribed sequence or not.

Pilocarpine treatment of fourth instar larvae was performed by adding the drug directly to the cultivation water. After several minutes, movement of the animals became uncoordinated. All salivary glands dissected after treatment times >3 h had morphologies consistent with an emptying of the lumen. Chromosome squashes revealed that Balbiani rings 1 and 2 in the majority of these glands appeared to be larger than normal. The Balbiani rings 1 and 2 increased in size over time reaching a maximum at ~7 h. Double-labeling immunofluorescence experiments were done with polytene chromosomes from untreated larvae and those treated for various lengths of time (Figure 5). In each case, antibody to RNA polymerase II stained the entire Balbiani ring evenly. In the absence of drug treatment, the staining pattern of the B52 antibody appeared identical to that of the RNA polymerase II antibody. After 5 h of treatment, slight differences in the staining pattern of the two antibodies could be seen at the periphery of the ring. At 7 h, staining of the large rings by the two antibodies was dramatically different. The RNA polymerase II antibody continues to stain the ring evenly, but the B52 antibody staining is concentrated on the branched condensed chromatin in the center of the ring. Therefore, at the highest level of transcription and decondensation of the Balbiani rings, the B52 distribution resolves from that of RNA polymerase II.

B52 on *Drosophila* Heat Shock Puffs and *Chironomus* Balbiani Rings Is Concentrated on the Branching Network of Chromatin Fibers

After 7 h of pilocarpine treatment, both the B52 antibody and 4,6-diamidino-2-phenylindole (DAPI) stained most intensely the branching chromatin fibers of the Balbiani rings (Figure 5). The branched fibers visible by DNA staining in the Balbiani rings is composed of folded chromatin, into 30-nm fibers or larger fibers (Ericsson *et al.*, 1989).

Staining of *Drosophila* heat-shock puffs with B52 antibody revealed that B52 is also localized to regions vis-

Figure 4. Distribution on *Chironomus* polytene chromosomes of proteins cross-reactive with *Drosophila* B52 antibodies and RNA polymerase II antibodies. *Chironomus* salivary gland polytene chromosomes from fourth instar larvae were fixed, spread, and incubated with both affinity-purified *Drosophila* B52 mAbs and polyclonal antibodies on salivary gland polytene chromosomes from fourth instar larvae. The binding of antibodies was visualized by indirect immunofluorescence. The same chromosomes were stained in A with affinity-purified B52 mAb and a secondary goat anti-mouse antibody conjugated to rhodamine, and in B with affinity-purified B52 polyclonal antibody and a secondary goat anti-rabbit antibody conjugated to fluorescein. Bar, 20 μ M. Arrows identify Balbiani Rings 1 and 2, where Ring 1 is closest to the end of the chromosome. *Chironomus* salivary gland polytene chromosomes from fourth instar larvae were also fixed, spread, and incubated with *Drosophila* B52 and RNA polymerase antibodies. (C) Phase contrast image. (D) Staining by affinity-purified *Drosophila* B52 mAb and a secondary goat anti-mouse antibody conjugated to rhodamine. (E) Staining by affinity-purified RNA polymerase II antibody and a secondary goat anti-rabbit antibody conjugated to fluorescein. Bar, 20 μ m.

ible by staining of the DNA with DAPI (Figure 6). These regions frequently appear to consist of somewhat branched chromatin fibers. Therefore, proteins cross-reactive with B52 antibodies in both *Drosophila* and *Chironomus* appear to be localized to the border between the decondensed regions of highly transcribed chromatin and the flanking, inactive, and condensed chromatin.

DISCUSSION

B52 and RNA polymerase II exhibit very similar distributions on polytene chromosomes and both are recruited to newly activated loci (Champlin *et al.*, 1991). However, at highly active heat-shock loci the proteins begin to resolve. In this paper, we have examined how B52's distribution on genes varies with changes in the

Figure 5. Distribution of B52 and RNA polymerase II cross-reactive proteins on chromosomes from pilocarpine-induced larvae. Double-label indirect immunofluorescence of *Chironomus* polytene chromosomes was done as in Figure 4. Each row shows the same chromosome from larvae treated with pilocarpine for the stated length of time. For the 7-h time point, it appears that the tip of the fourth chromosomes immediately distal to Balbiani ring 1 was twisted during squashing and is perpendicular to the focal plane. After photographing the immunofluorescence images, the slides were stained for 5' with 10 ng/ml DAPI as in Champlin *et al.* (1991). Bar, 20 μ m.

density of transcription complexes. First, we have heat shocked cells at different temperatures to vary systematically the density of transcription complexes on heat-shock genes and examined the resulting B52 distribution at high resolution by in vivo protein/DNA cross-linking. Second, we have examined the distribution of a likely homologue of B52 within developmentally active puffs in *Chironomus* by immunofluorescence both before and after hyperinduction of these puffs with the drug pilocarpine. These two complementary approaches demonstrate that the level of transcription determines the chromatin distributions of *Drosophila* B52 and the cross-reactive 46-kDa *Chironomus* protein. B52 is recruited to heat shock loci even when they are transcribed at low levels. As transcription is increased, the distribution of the protein across the locus changes, ultimately becoming concentrated at the borders of the region when maximum density of RNA polymerase II is reached.

Both the mAbs and polyclonal *Drosophila* B52 antibodies identify proteins in *Drosophila* and *Chironomus* of similar size and chromosomal distribution. As in *Drosophila*, the 46-kDa *Chironomus* protein generally

appears to be localized to transcriptionally active regions. Double-labeling of chromosomes from pilocarpine-stimulated larvae, however, reveals that although the anti-RNA polymerase II antibody stains the entire Balbiani ring homogeneously, the anti-B52 antibody mainly stains the branched condensed chromatin in the center of the ring just as would be predicted for a B52 homologue. A reexamination of large *Drosophila* heat-shock puffs reveals that the regions of B52 localization at the edge of the puff also appear to include branched condensed chromatin. The ultrastructural differences between *Drosophila* heat shock puffs and *Chironomus* Balbiani rings give rise to different images for B52 proteins that are localized to apparently homologous positions on the chromosomes. The Balbiani ring results provide additional evidence that the bracketing seen in *Drosophila* puffs is not an artifact of preparation or staining. The flexible distributions of the proteins are consistent with a dynamic model in which the B52 proteins track the condensation or the decondensation of chromatin.

B52 is located at regions of chromatin where condensation or decondensation is known to occur. In the

Figure 6. Distribution of B52 relative to DNA on a *Drosophila* heat-shock puff. Indirect immunofluorescence of B52 on salivary gland polytene chromosomes from heat-shocked third instar *Drosophila melanogaster* larvae was done as described previously (Champlin *et al.*, 1991). The same puff is stained with affinity-purified B52 mAb and a secondary goat anti-mouse antibody conjugated to rhodamine in A and with Hoechst 33258 (Somerville, NJ) in B. The heat shock puff is at 98B in the transformant line cHBD-89 that contains an *hsp70-lacZ* gene at this locus (Simon *et al.*, 1985). Arrows show an area overlap of B52 and branched chromatin fibers that bridge the region between the most decondensed DNA of the puff and the condensed DNA of the flanking polytene band.

case of highly induced heat-shock genes, transitions in chromatin organization are expected to be restricted to the boundaries of the highly active domain; whereas, at other loci (most genes are transcribed at a much lower frequency than heat shock genes) (Gilmour and Lis, 1985), these transitions reoccur continuously throughout the transcribed region as each RNA polymerase passes and chromatin refolds in its wake (Bjorkroth *et al.*, 1988). The short stretch of DNA between the *hsp23* and *hsp26* genes appears to be packaged in nucleosomes during full heat shock, whereas the active genes themselves appear in a more extended configuration (Cartwright and Elgin, 1986; Nacheva *et al.*, 1989). Because B52 does not cross-link well to restriction fragments spanning this region, it seems unlikely that B52 recognizes the transition between nucleosome and highly decondensed chromatin of the fully active heat-shock genes. Rather, B52 may localize to the boundary of a higher level of chromatin organization, perhaps the 30-nm fiber, that is expected to be present at the borders of the

small heat shock gene cluster. The differences in the distribution of B52 at loci containing moderately or highly expressed heat-shock genes are consistent with the view that the boundaries of highly transcribed chromatin identified by B52 are not static but rather are in a dynamic equilibrium between condensation and decondensation.

The sequence of the *Drosophila* B52 cDNA exhibits striking homology with the human splicing factor ASF/SF2 and SC35 (Champlin *et al.*, 1991; Ge *et al.*, 1991; Krainer *et al.*, 1991; Fu *et al.*, 1992). *Drosophila* B52 shows 52% identity over 233 of the 248 amino acids of the human ASF/SF2 protein. This includes a very good match to the two copies of the RNA recognition motif in the N-terminal portion and presence of multiple arrays of Ser-Arg repeats in the C-terminal portion of the protein. Moreover, *Drosophila* B52 can substitute for human ASF/SF2 in vitro, providing both the essential splicing activity of ASF/SF2 and the ability to influence 5' splice choice in a concentration-dependent manner (Zuo, Lis, and Manley, unpublished data), as can SRp55, a nearly identical *Drosophila* variant of B52 (Roth *et al.*, 1991; Mayeda *et al.*, 1992).

The distribution of several other proteins involved in packaging and splicing of pre-mRNAs (e.g., protein of U1snRNP and hnRNPs) have been examined. The location of several have been examined on heat-shock puffs and Balbiani rings, and the distributions of these proteins are indistinguishable from that of RNA polymerase II (Christensen *et al.*, 1981; Risau *et al.*, 1983; Sass and Pederson, 1984; Martin *et al.*, 1987; Vazquez-Nin *et al.*, 1990). On highly transcribed genes, both B52 and the *Chironomus* 46-kDa protein are concentrated at positions distinct from that which might be expected of a component of transcription or RNA processing. Also, unlike hnRNPs, this distribution of B52 on polytene chromosomes is not erased by RNAase treatment (Champlin *et al.*, 1991; Krause and Lis, unpublished data). In this regard, the properties of B52 resemble those reported of the mammalian SC35 protein (Spector *et al.*, 1991), which is structurally and functionally related to B52/ASF/AF2. SC35 is found in a speckled pattern in human interphase nuclei. Although the relationship of these speckles to the puff border labeling seen in *Drosophila* polytene chromosomes has not been directly addressed, both reside at or very near to sites of active transcription and RNA processing (Carter *et al.*, 1993). SC35's association with speckles is not altered by RNAase treatment (Spector *et al.*, 1991). Additionally, SC35 can redistribute near to newly activated regions of the nucleus as seen clearly during adenovirus infection (Jimenez-Garcia and Spector, 1993), a property that may be related to B52's redistribution to the activated heat shock loci as seen here and in Champlin *et al.* (1991). It is also of interest that the most intensely labeled SC-35 speckles in interphase nuclei resolve from the sites of intense pre-mRNA synthesis (Wansink *et*

al., 1993). Therefore, the *Drosophila* B52 and *Chironomus* 46-kDa proteins and their mammalian homologues are likely to play roles that are spatially and functionally distinct from that of most components of the transcriptional or RNA processing machinery.

Why would a protein that appears to function as an essential component of splicing and a regulator of alternative splice site choice track to changes in chromatin structure? Such changes occur on genes with every passage of RNA polymerase II and could concentrate B52 at or near to sites where its RNA substrate is being made. Additionally, if B52 interacted with the RNA or splicing machinery yet managed to continue to track with changes in chromatin structure caused by the transcribing RNA polymerase, then it could deliver a 5' splice site to the next 3' splice site encountered by RNA polymerase. The splicing need not follow immediately after B52 recognizes the 5' splice site, but at this point a commitment to splice may be made. Such a coupling of transcription and splicing would provide a mechanism for the ordered joining of exons that would be independent of intron length and could conceivably act over the 100-kb introns present in some genes.

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