The location and dynamics of small nuclear ribonucleoproteins (snRNPs) were studied in salivary gland polytene chromosomes of Chironomus tentans by immunofluorescence with specific snRNP antibodies. Monoclonal antibody against the snRNP Sm antigens reacted at all sites of transcription (puffs and Balbiani rings). The amount of snRNP immunofluorescence was strictly dependent on transcription, increasing in parallel with gene activation and decreasing upon repression. Identical patterns of localization and transcriptional dependence were observed with antibodies specific for U1 or U2 snRNPs. These latter results show that the involvement of U1 and U2 snRNPs in transcription-related processes involves a high proportion, rather than small subsets, of active gene loci. In addition, the colocalization of U1 and U2 snRNPs at loci known to contain only one messenger RNA transcription unit (e.g. Balbiani ring 2) raises the possibility that both of these snRNPs interact with the same transcript. Finally, the lack of immunofluorescence at repressed loci indicates that snRNPs are not structural components of the chromatin (DNP) fiber, and also shows that unused snRNPs are not stored in chromatin. These latter points, and the growing evidence for the involvement of U1 snRNP in splicing, suggest that nascent pre-mRNA is the major chromosomal binding site for snRNPs.

1. Introduction

Most eukaryotic messenger RNAs are transcribed as precursor molecules that undergo subsequent processing reactions, including base and ribose methylations, trimming of the 3' end, poly(A) addition and splicing. The latter reaction appears to involve the participation of a small nuclear ribonucleoprotein, U1 RNP§ (Lerner et al., 1980; Rogers & Wall, 1980; Calvet & Pederson, 1981; Yang et al., 1981; Mount et al., 1983; Hernandez & Keller, 1983; Padgett et al., 1983; Setyono & Pederson, 1984). U1 RNP is one of several abundant, evolutionary conserved snRNPs.
small nuclear RNA–protein complexes currently being investigated as possible cofactors in messenger RNA processing.

The specificity of certain human autoantibodies for snRNPs (Lerner & Steitz, 1981) has accelerated the study of both their RNA and protein components. In addition, an autoantibody specific for U1 RNP has provided a means to probe its function using in vitro mRNA splicing extracts (Yang et al., 1981; Padgett et al., 1983). Genetic approaches to snRNP function in vivo are also available in organisms, such as yeast, in which the RNA components of snRNPs are encoded by single-copy genes (Wise et al., 1983; Tollervey et al., 1983). A third avenue to the elucidation of snRNP function is to examine their interaction with individual chromosomal loci in polytene chromosomes, by immunofluorescence with specific autoantibodies. This approach can, in principle, resolve such issues as: (1) whether snRNPs are structural components of the chromatin (DNP) fiber; (2) whether the presence of snRNPs at specific loci is temporally coupled with transcription; and (3) whether different species of snRNP have a similar or non-overlapping chromosomal distribution. In this paper we report a detailed investigation of snRNP localization in well-characterized, isolated polytene chromosomes of *Chironomus tentans* (Sass, 1980a, 1982), the results of which provide answers to these questions.

### 2. Materials and Methods

(a) **Animals and cells**

Larvae from inbred laboratory strains of *Chironomus tentans* (Fabr.) were used. Egg masses and larvae were originally provided by Dr R. Tanguay (University of Laval, Quebec, Canada). The animals were raised in our laboratory as described (Sass, 1981). Larvae of the chironomid *Glyptotendipes barbipes* taken from natural habitats in Pennsylvania were obtained from C.R.A.W.-Fish, Worcester, MA. HeLa cells were from our standard laboratory stock and grown in suspension cultures as described (Pederson, 1972).

(b) **Autoantibodies**

Monoclonal anti-Sm antibody was prepared from ascites fluid of Pristine-primed BALB/c mice as described (Lerner et al., 1981). Anti-(U1)RNP and anti-La sera were obtained from patients with mixed connective tissue disease and Sjögren’s syndrome, respectively, and have been described (Wieben et al., 1983a; Madore et al., 1984a,b). Anti-(U2)RNP serum from a patient with scleroderma-polymyositis overlap syndrome (Mimori et al., 1984) was kindly provided by T. Mimori (Yale University). Immunoglobulin G (IgG) was prepared from patients sera as described (Douvas et al., 1979). Control non-immune IgG was obtained from sera of healthy laboratory personnel.

(c) **Preparation of radiolabeled larval homogenates and cell nuclear extracts**

Fifty or 100 fourth instar larvae (0.65 g or 1.3 g weight) of *G. barbipes* or *C. tentans* were labeled in 5 ml of a solution containing 0.06% (w/v) NaCl, 0.005% (w/v) MgCl₂, 0.005% (w/v) CaCl₂ plus 5 mCi [³H]uridine for 17 to 34 h at 18°C, with aeration. After labeling the larvae were rinsed 3 times with ice-cold 150 mM-NaCl, buffered at pH 8.3 with 10 mM-Tris-HCl. Larvae were immediately homogenized with a motor-driven teflon–glass Potter Elvehjem homogenizer in 2 ml of homogenization buffer (100 mM-NaCl, 1 mM-MgCl₂, 1 mM-dithiothreitol, 1000 units ribonuclease inhibitor RNasin/ml (Promega...
SMALL NUCLEAR RNPs AT ACTIVE GENE LOCI 913

Note(>). 0.2%, (v/r) diethylpyrocarbonate, 1 mM-phenylmethyl sulfonyl fluoride. 2 µg trypsin inhibitor from soybean/ml (Serva). 10 mM-Tris-HCl (pH 8.3). The homogenate was then sonicated (4 × 30 s at setting 3; Heat Systems Ultrasonics model W 375) and centrifuged for 15 min at 8000 revs/min at 2°C in a Sorvall HB-4 rotor. The supernatant was used for antibody precipitation of RNPs. In some experiments, the salivary glands of labeled larvae were dissected and homogenized by sonication (30 to 60 s at setting 7) in homogenization buffer. Salivary gland homogenates were centrifuged for 5 min in an Eppendorf microfuge and the cleared supernatant was used for antibody precipitation. HeLa cell nuclear extracts were prepared using slight modifications of previously published methods (Lerner & Steitz, 1979; Lerner et al., 1981; Wieben & Pederson, 1982).

(d) Immunoprecipitation of RNPs containing snRNAs and gel electrophoresis

RNPs were immunoprecipitated from larvae and salivary gland extracts by adding 100 µg of IgG to 500 µl of extract and incubating for 30 min on ice. 200 µl of protein A-Sepharose (CL-4B, Pharmacia) in 150 mM-NaCl, 0.05% (v/v) NP 40 (Particle Data Laboratories Ltd, Elmhurst, IL U.S.A.), 50 mM-Tris-HCl (pH 8.3) were then added for an additional 15 to 30 min. The protein A-Sepharose was pelleted by centrifugation for 40 s in a microfuge and washed repeatedly with NET-2 (Kessler, 1975). After elution with 0.1 M-glycine (pH 3.0), the eluate and in some cases also the NET-2 washes were extracted with phenol/chloroform/isoamylalcohol (50 : 50 : 1, by vol.) containing 0.1% (w/v) 8-hydroxyquinoline and 1% (w/v) sodium dodecyl sulfate. RNA was precipitated with 2 vol. ethanol and fractionated by electrophoresis in 10% (w/v) polyacrylamide gels containing 7 M-urea, 90 mM-Tris-borate (pH 8.3), 2 mM-EDTA. Gels were fluorographed with En3Hance (New England Nuclear) and exposed to preflashed Kodak XAR film at −70°C.

(e) Chromosome isolation

Salivary gland chromosomes of fourth instar larvae of C. tentans and G. barbipes were isolated in a native condition as described in detail by Sass (1980a, 1982, 1984).

(f) Transcription autoradiograms

Larvae were injected orally with [3H]uridine according to the method of Sass (1980a). Alternatively, dissected salivary glands were labeled as described (Sass, 1981). In both cases the duration of labeling was 5 min. The chromosomal preparations were exposed to Kodak AR lo-stripping film for 3 weeks.

(g) Immunofluorescence

Isolated salivary gland chromosomes of C. tentans and G. barbipes were prepared for antibody reactions as described (Sass, 1982). Chromosomes were then incubated at room temperature with 10 µl mouse monoclonal anti-Sm antibody or one of the human antisera diluted 1 : 10, 1 : 30, 1 : 50 or 1 : 100 in PBS (phosphate-buffered saline). After washing in PBS for 3 × 10 min the chromosomes were incubated for 30 min at room temperature with FITC-conjugated rabbit anti-mouse IgG or rabbit anti-human IgG (Cappel), diluted 1 : 50 or 1 : 100 in PBS. In controls, identical procedures were used except the primary antibody was replaced by a 30 min wash in PBS.

3. Results

(a) Chironomid small RNPs react with antibodies for mammalian snRNPs

To investigate the reactivity of chironomid small RNPs with mammalian snRNP antibodies we used fourth instar larvae of Glyptotendipes barbipes,
FIG. 1. Specificities of Sm, RNP and La antibodies in chironomid larvae. Immunoprecipitations of RNP's from extracts of [3H]uridine-labeled larvae of the chironomid Glyptotendipes barbibes and HeLa cell nuclei were performed as described in Materials and Methods. Antibody-selected RNA was analyzed in 10% (w/v) polyacrylamide gels containing 7 M-urea. Lane 1, total RNA of chironomid extract; lanes 2, 3 and 5, HeLa nuclear extract, anti-Sm; lanes 4 and 11, chironomid extract, anti-Sm; lane 6, chironomid extract, anti-La; lane 7, chironomid extract, anti-U1 RNP; lane 8, extract of larvae from which salivary glands had been removed, anti-Sm; lane 9, chironomid larvae extract, non-immune human IgG; lane 10, salivary gland extract, anti-Sm; lane 12, RNAs present in NET wash of protein A-Sepharose after reaction of Sm antibody with chironomid larvae extract.

available to us in large quantities, whereas we used its close relative C. tentans (Keyl & Keyl, 1963; Walter, 1973) for the immunofluorescence studies. Figure 1 shows that antibodies for mammalian snRNPs cross-react with small nuclear RNPs of chironomid whole larval extracts. Monoclonal Sm antibody was found to react with RNPs containing several small RNA species as shown in lane 4. The most abundant of these were termed U2 and U1 by virtue of their similar electrophoretic migration with human U2 and U1 RNAs (lane 3), and the
hybridization of the latter with a *Drosophila* U1 DNA probe (not shown). A third chironomid RNA comigrated with human U4 RNA. Also present in the Sm-reactive chironomid RNPs was a RNA species of approximately 400 nucleotides (arrow, lane 4). However, this component was not consistently observed and may be related to the physiological condition of the larvae (preliminary results). Comparison of the antibody-selected RNAs with total larval small RNA (lane 1) revealed the extreme selectivity of the monoclonal Sm antibody reaction. The total small RNA pattern (lane 1) is considerably more complex than that of mammalian small nuclear RNAs (e.g. Lerner & Steitz. 1979). This agrees with a similar situation in *Drosophila* (Wieben & Pederson, 1982). Human (U1) RNP-specific autoantibody reacted selectively with chironomid RNPs containing U1 RNA (lane 7). La autoantibody did not react with any small RNPs (lane 6).

As the immunofluorescence data to be presented were obtained with salivary gland chromosomes, we checked the remote possibility that the antibody-reactive small RNPs in this tissue differ from those of whole larval extracts. As shown in lane 10 of Figure 1, Sm antibody reacted with a set of salivary gland small RNPs having the same electrophoretic pattern of RNAs as those from whole larvae (lanes 4 and 11), and from larvae from which salivary glands had been removed prior to homogenization (lane 8).

(b) Localization of Chironomus small nuclear RNPs at loci of gene transcription in polytene chromosomes

To investigate the possible relationship between sites of gene transcription and the presence of snRNP, salivary gland chromosomes manually isolated from *C. tentans* larvae were analyzed by indirect immunofluorescence with Sm monoclonal antibody. In parallel, levels of transcription were monitored by autoradiography of chromosomes in squash preparations from larvae labeled with $[^3]H$uridine.

The most active transcription sites in larval salivary glands of *C. tentans* are three Balbiani rings (BR1, BR2 and BR3) on chromosome IV (Fig. 2(a)). These loci consistently contained the highest concentrations of snRNPs, as revealed by immunofluorescence with Sm monoclonal antibody (Fig. 2(d)). This observation was made in 100% of 138 chromosomes examined. The less active sites of transcription appeared after antibody staining as traverse bands of low, variable fluorescence intensity. These sites, which are the typical *Chironomus* puffs, thus contain lower amounts of snRNPs (Fig. 2(d)). The regions between these immunofluorescent puffs were almost completely devoid of antibody staining, a point to which we shall return. The Sm antibody staining patterns were identical when salivary glands were briefly fixed just prior to chromosome preparation as when fixation was performed on isolated chromosomes. No labeling was detected with second antibody alone (not shown).

In order to further explore the correlation between transcription and snRNP presence, we used dimethylsulfoxide treatment and heat shock to modulate gene activity of distinct loci (Sass, 1980a,b,1981,1982). Exposure of fourth instar larvac
to 10\% Me₂SO for three to four hours stimulates BR1, BR2 and BR3 transcription (Fig. 2(b)). Sm antibody staining also revealed highly expanded snRNP concentrations at BR1-3 after Me₂SO treatment in 84 out of 84 cases examined (Fig. 2(e)). This indicates that a relationship exists between
transcriptional activity of specific chromosomal loci and the amount of snRNPs present.

Heat shock (37° to 39°C, 50 min) leads to regression of Balbiani rings and the activation of two puffs at loci 2B and 5C on chromosome IV (Sass, 1980a, 1982; Lezzi et al., 1981), as shown in Figure 2(c). These two heat shock puffs, and also other heat-stimulated loci on chromosomes I to III, stained prominently with Sm antibody, while the regressed Balbiani rings and other loci stained weakly (Fig. 2(f)). Again, this demonstrates a correlation between transcription and the presence of snRNPs.

Another modulation of gene transcription in C. tentans salivary gland chromosomes occurs during the recovery of larvae from Me₂SO treatment. The stimulated Balbiani rings collapse within 30 minutes after transfer of larvae to medium without Me₂SO. BR1 and BR3 resume intense transcription over the next one to two hours, while BR2 remains repressed (Sass, 1981). This situation therefore provides an excellent opportunity to determine, in the absence of Me₂SO or heat shock, the extent to which the presence of snRNP is correlated with transcription. Figure 3(a) and 3(d) illustrate, respectively, the transcriptional activity and snRNP localization on chromosome IV in untreated larvae. Figure 3(b), (c), (e) and (f) show recovery from Me₂SO treatment. Thirty minutes to one hour after removal of Me₂SO, the resumption of transcriptional activity at BR1 and BR3 (Fig. 3(b)) was correlated with intense Sm antibody staining, whereas the still-repressed BR2 exhibited only a trace of immunofluorescence (Fig. 3(e)). After two hours, transcriptional activity and Sm immunofluorescence further increased in parallel at BR1 and BR3, while BR2 still remained relatively inactive (arrow, Fig. 3(c)) and very weakly immunofluorescent (arrow, Fig. 3(f)). The pattern shown in Figure 3(f) was consistently observed on chromosome IV in each of 75 chromosome IV preparations. This result thus demonstrates that snRNP presence and transcriptional activity are correlated in the absence of Me₂SO or heat shock.

In order to explore the presence of snRNP in typical puffs, we analyzed chromosome I, which contains a large number of small, typical puffs and is devoid of Balbiani rings. Sm antibody stained an average of 80 discrete sites on chromosome I (Fig. 4(a)), including heat shock locus 1-20A, which is known to be active under normal conditions. This number of immunofluorescent puffs agrees with the number of [³H]uridine-labeled puffs on this chromosome (Sass, 1980a, b). Prolonged Me₂SO treatment gradually represses transcription of chromosome I and II to IV (Sass, 1981). This was correlated with a large decrease in Sm antibody staining, with the exception of the newly stimulated locus 1-4C (Fig. 4(b) and (c)).

c) Distribution of U1 and U2 RNPs at stimulated and repressed gene loci

The relative contributions of U1 RNP and U2 RNP to the Sm antibody immunofluorescence patterns were investigated using autoantibodies specific for each of these two RNPs. The staining pattern of chromosome IV with U1 RNP-specific antibody was identical to that observed with Sm antibody, with BR1-3
Fig. 3. Effects of directed changes in transcriptional activity on Sm immunofluorescence in absence of drugs or physiological stress. Chromosome IV \( ^{3} \text{H} \)uridine transcription autoradiograms and parallel Sm immunofluorescence are shown for: (a) and (d) untreated larvae; (b) and (e) larvae returned to regular medium for 20 to 60 min following 8 to 10 h of \( 10\% \) Me\(_2\)SO treatment; (c) and (f) larvae returned to regular medium for 2 h after Me\(_2\)SO treatment as in (b) and (e). The modulated BR2 locus is indicated by the arrows in (b), (c), (e) and (f).

again comprising the major reaction sites (Figs 5(a) and 6(a); compare with Sm pattern in Fig. 2(d). Selective transcriptional repression of BR2 by Me\(_2\)SO treatment and reversal resulted in a nearly complete absence of U1 snRNP from BR2, while the active BR1 and BR3 loci were stained intensely (Fig. 5(b); compare with the transcriptional activities of BR1-3 in Fig. 5(d)); arrows in both panels indicate BR2). The correlation between U1 snRNP and transcription is further exemplified by the results shown in Figure 6. Immediately after Me\(_2\)SO treatment (30 min of reversal) the retraction of BR1-3 and suppression of transcription (Fig. 6(c)) was accompanied by greatly reduced amounts of U1 snRNP (Fig. 6(b)). Heat shock also led to a reduction of U1 snRNP, except at the heat shock-induced puffs IV-2B and IV-5C (Fig. 6(d); compare with the Sm
antibody pattern in Fig. 2(f)). Me$_2$SO treatment and heat shock also resulted in closely correlated patterns of U1 snRNP localization and transcriptional activity on chromosomes I to III (not shown). The chromosomal level of U1 snRNP behavior was in all respects indistinguishable from that of the snRNPs recognized by Sm antibody.

Fig. 5. U1 RNP immunofluorescence and transcription of stimulated and repressed Balbiani rings. (a) Immunofluorescence with anti-U1 RNP of chromosome IV from an untreated larva; (b) immunofluorescence after treating larva for 6 h with Me$_2$SO; (c) phase-contrast image of the same chromosome as in (b); (d) $[^3]$H]uridine autoradiogram of chromosome IV from a larva treated with Me$_2$SO as in (b). Arrows in (b) to (d) indicate the repressed BR2 locus.
An autoantibody specific for mammalian U2 snRNP was shown to react only with U2 snRNP in Drosophila Kc cells (Mimori et al., 1984). This antibody stained C. tentans chromosome IV in a pattern indistinguishable from that of Sm and anti-U1 RNP antibodies (Fig. 7; compare with Figs 2(d), 3(d), 5(a) and 6(a)). The staining patterns observed with 1 : 100 to 1 : 500 dilutions of the anti-U2 snRNP serum were the same, suggesting that the reaction is U2 snRNP-specific (Mimori et al., 1984). Experimental modulation of transcription by MeSO or heat shock led to the same alterations of U2 RNP-specific antibody immunofluorescence as those observed with Sm and anti-U1 RNP antibodies.

(d) Analysis of snRNP localization in chromosomal subdivisions

In establishing a relation between transcription and snRNP presence, emphasis was placed on major transcription sites, e.g. BR1-3 and certain heat shock loci. The following experiments address the localization of snRNP in chromosomal subdivisions: bands, interbands and puffs. This issue was investigated by detailed analysis of mechanically stretched chromosomes. This procedure increases the resolution of bands and interbands but does not change band number per chromosome (Beermann, 1972). Figure 8(a) and (b) shows a stretched chromosome IV stained with Sm monoclonal antibody (Fig. 8(a)) compared with the phase-contrast image (Fig. 8(b)). In addition to the familiar Balbiani rings, other less prominent loci (e.g. IV-2C, -4C, -5A, -5C) were stained. However, many other loci were stained weakly or not at all. Similar patterns were observed for U1 and U2 RNPs using the respective specific antibodies (Fig. 9). Comparison of the immunofluorescence patterns, obtained with all three antibodies, with parallel
phase-contrast images revealed a general correlation between band structure and amount of snRNP present. Most of the moderately to strongly stained loci appeared in phase-contrast as loosened bands (“LB”; Figs 8 and 9). We regard these immunofluorescent loci as puffs because their locations correspond to those previously mapped by \(^{3}\text{H}\)uridine incorporation (Pelling, 1964). Characteristically, typical *Chironomus* puffs cannot be easily distinguished from the more condensed bands by phase-contrast microscopy alone (Beermann, 1962, 1972; Pelling, 1972), and this is more pronounced in isolated chromosomes (Sass, 1980a). Finally, the interband regions were consistently devoid of staining by Sm, U1 RNP and U2 RNP antibodies (Figs 8 and 9). The presence of snRNPs on puff-like, loosened bands and their absence from interbands therefore serves to further establish the concordance between snRNPs and transcription-related processes.

4. Discussion

(a) Cross-reaction of chironomid small nuclear RNPs with mammalian autoantibodies

We find that human autoantibodies and a mouse monoclonal antibody for snRNPs react with small RNPs of chironomid insects, both in cell extracts and on polytene chromosomes. This extends previous data showing that insect small nuclear RNAs are complexed with proteins cross-reactive with human RNP and Sm autoantibodies (Lerner *et al.*, 1980; Mount & Steitz, 1981; Wieben & Pederson.
The results of this study demonstrate a correlation between the transcriptional activity of individual chromosomal loci (puffs and Balbiani rings) and the amounts of snRNP present. The fact that snRNPs are present only at transcribed...
loci and were never observed in other chromosomal regions excludes a structural role in chromatin (DNP). Upon experimental repression of transcription, snRNP antibody staining of formerly active loci decreases in parallel with rates of \(^{3}H\)uridine labeling (Figs 2 to 6). This shows that inactive snRNPs are not stored in chromatin.

We interpret the immunofluorescence data as reflecting an association of snRNPs with heterogeneous nuclear RNA (pre-mRNA). This interpretation is compatible with previous demonstrations of snRNAs and/or snRNPs in hnRNP particles (Deimel et al., 1977; Northemann et al., 1977; Guimont-Ducamp et al., 1977; Flytzanis et al., 1978; Howard, 1978; Gallinaro & Jacob, 1979, 1981; Lerner et al., 1980; Zieve & Penman, 1981) and with evidence for base-pairing between U1 and U2 RNAs and hnRNA (Calvet & Pederson, 1981; Calvet et al., 1982; Setyono & Pederson, 1984). Ultrastructural and biochemical studies reveal that *Chironomus* Balbiani ring 2 contains predominantly nascent RNA and that the
completed transcripts are rapidly detached from the chromatin (Daneholt, 1972; Egyházi, 1976; Anderson et al., 1982). Accordingly, our results indicate an association of snRNPs with nascent hnRNA, at least in Chironomus polytene chromosomes. This raises the possibility that snRNPs begin to function on nascent hnRNA. Evidence for processing of Drosophila embryo nascent hnRNA chains has been presented (Beyer et al., 1980, 1981).

Data from in vitro systems strongly suggest that U1 RNP is involved in mRNA splicing (Mount et al., 1983; Hernandez & Keller, 1983; Padgett et al., 1983). The localization of U1 RNP on polytene chromosomes is therefore of considerable interest. We find that all major sites of transcriptional activity contain U1 RNP. This indicates that U1 RNP participates in mRNA processing at a very high proportion of the active gene loci, rather than being restricted to a small subset of pre-mRNA species. (We of course cannot exclude the presence of some pre-mRNA transcription sites that are negative for U1 RNP staining.) It is also pertinent to consider the relationship between U1 RNP localization and intron-containing gene loci. One of the major sites of transcription and of high U1 RNP concentration is Balbiani ring 2 (Figs 5 and 6). The biochemical investigation of BR2 transcripts has not revealed evidence for splicing (Case & Daneholt, 1978) although the presence of introns comprising ~5% or less of the primary transcript length cannot presently be ruled out. The strong U1 RNP antibody staining of heat shock-induced puffs (Fig. 6) is noteworthy in the same regard. Most Drosophila heat shock genes lack introns (Holmgren et al., 1979; Corces et al., 1980), although no information is available on this point for Chironomus. These considerations raise the possibility that U1 RNP may be a constitutive element of transcript packaging, irrespective of the presence or absence of introns in the pre-mRNA. For pre-mRNAs with introns, this might require movement of the hnRNP–U1 RNP complex along the pre-mRNA to position the U1 RNA with the consensus sequences at splice junctions (e.g. see Sharp, 1981).

The immunofluorescence pattern of U2 RNP localization was identical to that of U1 RNP under all conditions investigated. This suggests the possibility that both RNPs interact with the same pre-mRNAs or, alternatively, that both U1 and U2 RNPs are invariant constituents of hnRNP regardless of their deployment in splicing. U2 RNP does not bind to protein-free pre-mRNA in vitro under conditions where U1 RNP does (Mount et al., 1983). Perhaps there is cooperative binding of both U1 and U2 RNP to the same transcript, or a dependence of U2 RNP binding on hnRNP structure. As it is now possible to isolate U1 and U2 RNPs (Hinterberger et al., 1983; Kinlaw et al., 1983), and also to assemble specific hnRNPs in vitro (Economidis & Pederson, 1983), it should be feasible to investigate these issues with purified components.

(c) Bearing of snRNP distribution on gene location in polytene chromosomes

A long-standing question in the field of polytene chromosome structure and function is the location of genes, both as genetic and transcriptional elements, in relation to cytological units of organization. Recently, the issue of whether genes are present in interbands has arisen, partly due to observations of RNA polymerase II-mediated transcription in these regions (reviewed by Zhimulev
Our analysis of snRNP (with Sm monoclonal antibody) and U1 and U2 RNPs on stretched polytene chromosomes demonstrate that snRNPs exist exclusively in puffs and loosened bands. The latter represent minor puffs, or perhaps puffs in the process of activation or repression. The complete absence of snRNPs from interbands (Figs 8 and 9) strongly reduces the possibility that they produce pre-mRNAs. This argues against the proposal that interbands contain active gene copies transcribed by RNA polymerase II (Jamrich et al., 1977). However, the fact that in situ hybridization with a cloned DNA segment complementary to BR2 mRNA labels an interband-like region (Sass, 1984) indicates that an interband may contain a minor part of a gene. It is also possible that interbands are RNA polymerase II entry or start sites (Jamrich et al., 1977; Sass & Bautz, 1982a,b; Sass, 1982), with the majority of the transcription unit residing in the 3'-ward band. Our results do indicate, however, that the interband regions themselves are not significant sites of mRNA transcription in *Chironomus* polytene chromosomes.

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