Localization and expression of transformed DNA sequences within heat shock puffs of Drosophila melanogaster

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Abstract. In situ hybridization at high resolution with biotin-labeled DNA was used to locate specific transcriptional units within the chromosomal puffs of normal heat shock loci and of new heat shock loci generated by transformation. This method resolves copies of the hsp70 gene that are separated by 40 kb within the 87C heat shock locus. In the case of two new puff loci generated by transformation, the heat-activated transcript is encoded by sequences that reside entirely within the puff domain and the activated promoter is positioned asymmetrically within the puff. However, an adjacent promoter and its transcriptional unit which do not appear to be induced by heat shock are also within the puff. These results support the idea that a chromosomal puff is a structure that facilitates or results from vigorous transcription, but that the structural change alone is insufficient to induce transcription.

Introduction

Specific regions of Drosophila polytene chromosomes undergo a dramatic structural change, termed puffing, in response to heat shock (Ritossa 1962) as well as during development (Ashburner 1972). The precise function of chromosomal puffs is unknown, but their formation at specific chromosomal sites correlates with high transcriptional activity (Pelling 1964; Tissieres et al. 1974; Sass 1982). Three distinct hypotheses to explain the relationship between puffing and transcription may be considered. Firstly, puffing may play a pretranscriptional role in gene regulation such that the change in chromatin conformation triggers the subsequent transcriptional activation. Alternatively, puffing may simply be a consequence of vigorous transcription (Beermann 1966) and have no influence upon the initial steps of gene activation. Finally, a two-step process has been proposed (Berendes 1968; Daneholt et al. 1982) whereby puffing occurs to some extent as a precondition for transcription and then further decondensation accompanies transcription resulting in the completion of puff formation.

If puffing is indeed involved in gene activation then a question arises concerning differential regulation at puff loci: why are some genes which reside at puff loci not activated when the locus puffs? For example, the 63B heat shock locus of D. melanogaster contains the hsp83 heat shock gene and another transcriptional unit designated T2. Activation of the hsp83 gene accompanies puffing at this locus whereas transcription of T2 actually decreases even though both genes reside within the puff (O'Connor and Lis 1981). Similar differential regulation occurs at the 67B heat shock locus (Ireland et al. 1982). Genes that reside in regions prone to puffing but that resist activation when puffing occurs might have evolved special mechanisms which prevent coactivation. Alternatively, the process of puffing may be insufficient to function as a general activator of transcription.

The development of methods for Drosophila transformation (Rubin and Spradling 1982) makes possible a new approach toward addressing these questions concerning the relationship between puffing and transcription. Analysis of heat shock fusion genes introduced into novel chromosomal positions by transformation has shown that puffing does not require the structural environment provided by the normal locus (Lis et al. 1983; Bonner et al. 1984). Here we extend the analysis of such transformed puff loci by examining the DNA sequence organization within the puff using high resolution in situ hybridization methods that employ biotin-labeled DNA probes (Langer et al. 1981). In addition, we test for the heat shock activation of the rosy gene which, by this analysis, resides within these heat shock puffs.

Materials and methods

Salivary gland squashes were performed as described (Lis et al. 1978). Hybridization and detection were performed as follows: biotin-dUTP (Enzo-Biochem) was incorporated into DNA probes by nick-translation (Rigby et al. 1977). RNase A treatment just prior to denaturation did not affect the pattern of labeling obtained under the conditions employed here and therefore was omitted. Probe DNA at 3 ng/μl in hybridization buffer (10% dextran sulfate, 2×SSC, 50% formamide, 0.5 mg/ml E. coli tRNA and 0.5 mg/ml calf thymus DNA) was denatured at 75 °C for 10 min and 8 μl (24 ng) was applied directly over the squash. Hybridization conditions were as described (Lis et al. 1983). Slides were then washed three times, 30 min each, in 2×SSC (0.3 M NaCl, 0.03 M Na citrate) at 37 °C and four times, 5 min each, in PBS (0.13 M NaCl, 7 mM Na2HPO4 and 3 mM NaH2PO4) at room temperature. Then 20 μl each of avidin-DH and biotin-peroxidase (Vector Labs, Vectastain ABC Kit) were added to 1.0 ml of 4% w/v bovine serum albumin (BSA) in 50 mM Tris pH 7.5 and after prebinding for 5 min at room temperature, 100 μl...
was added to each slide. The slides were covered with a piece of paraffin cut to fit the slide and incubated at 37°C for 1 h. Slides were then washed in PBS, as above, and the staining mix was prepared by adding 2 mg of 3,3'-dianinobenzidine (Sigma) and 10 μl of 30% hydrogen peroxide to 4.0 ml of 50 mM Tris pH 7.5 at room temperature; 100 μl of this mix was added per slide and the reaction was allowed to proceed approximately 3 h at 37°C. After washing in PBS, chromosomes were stained with Giemsa (Fisher).

Adult fly RNA was prepared by grinding whole flies in 10 mM Tris pH 7.5, 20 mM EDTA, 1% sodium dodecyl sulfate (SDS), followed by two phenol extractions with equilibrated phenol and one phenol extraction using phenol:chloroform:isoamyl alcohol (50:50:1). After two ether extractions, RNA was recovered by ethanol precipitation. RNA was electrophoresed through 0.8% agarose, 1.1 M formaldehyde gels and transferred to nitrocellulose, essentially as described by Maniatis et al. (1982) except that 10 mM NaPi, pH 6.8, 1.1 M formaldehyde was used as electrophoresis buffer.

**Results and discussion**

A variety of cloned DNA segments (Fig. 1) were biotin-labeled (Langer et al. 1981) and hybridized in situ to chromosome preparations from heat-shocked *D. melanogaster* larvae containing additional heat shock loci generated by transformation (Lis et al. 1983). Hybridization was visualized using a peroxidase detection system. Figure 1c (arrow) shows that a compact band of signal at 87D is obtained with hybridization of a 4.6 kb EcoRI fragment from an 87D (rosy) genomic clone. In situ hybridization with probes to puffed regions of the chromosome can also be detected using this method, however the signal tends to be more diffuse.

The 87C shock locus of *D. melanogaster* contains three copies of the heat shock gene, hsp70, with the organization depicted in Figure 1a. The one proximal copy and two distal copies of the hsp70 gene are separated by approximately 40 kb (Ish-Horowicz and Pinchin 1980). In situ hybridization using a 1.3 kb probe which contains the 5' half of the hsp70 gene produces two distinct regions of labeling within the 87C puff (Fig. 1a, top). This pattern is consistent with the known physical map of 87C. This and several other lines of evidence indicate that this hybridization method can be used to reveal the sequence organization within a puff. Firstly, the distal labeling at 87C was reproducibly greater than the proximal labeling, presumably reflecting the presence of two distal gene copies versus one proximal copy. Secondly, examination of 60 such hybridized nuclei, in which bipartite staining at 87C was readily discernible, consistently revealed uniform staining of 87A, a locus which contains 2 copies of hsp70 separated by only 1.7 kb (Ish-Horowicz and Pinchin 1980). Thirdly, a middle repetitive element, ϱΦ (Lis et al. 1978), which at the 87C locus has been shown to reside solely in the region between the divergent copies of hsp70 (Ish-Horowicz and Pinchin 1980), hybridizes to the central region of the puff (Fig. 1a, bottom).

The hybridization signals within the puff are broader than expected if the sequences of each chromatid were aligned in register. Even in the extreme case, the length occupied by 1.3 kb of maximally extended B-form DNA is 1.3 × 0.34 μm or 0.44 μm [derived from Watson and Crick (1953)]. Yet the proximal band of hybridization within the 87C puff was found, on average, to extend 3 μm in length. The discrepancy cannot be attributed to limits in the physical resolution of the method since hybridization of even larger probes to nonpuffed regions regularly yields hybridization signals which extend for only 0.5–1 μm (for example, Fig. 1c). A more likely explanation is that the homologous sequences of individual chromosomal strands of the puffed polytene chromosome are not perfectly aligned but rather experience some staggering from one chromatid to the next.

Having established that we could investigate the in situ sequence organization of puff domains using this hybridization method, we addressed questions concerning the relationship between puff organization and transcription. We chose to focus on the 61A and 9E heat shock loci of line Bg9.61 (Lis et al. 1983) because of their large puff sizes, simple organization (one heat shock gene per puff) and defined physical maps. These heat shock loci were generated by P element mediated transformation (Rubin and Spradling 1982) with the plasmid ep19.1 (Lis et al. 1983) resulting in insertion of the entire segment depicted in Fig. 1b. The hsp70 promoter is located within the 200 bp region downstream from the XhoI site on the left. The rosy transcriptional unit is downstream of the hsp70-lacZ gene and both genes are oriented left to right. The major heat-inducible transcript derived from this insert is an approximately 11 kb species (Fig. 2a) that appears to initiate at the start of the hsp70 transcriptional unit and terminate within sequences upstream of the rosy gene (J. Simon, unpublished results).

In situ hybridization to the 61A and 9E puff loci is shown in Figure 1b. The 5' hsp70 probe (I) hybridizes to sequences within the puff domain yet the signal in both cases is detected close to one border of the puff. This result establishes that, at least in these cases, the heat shock promoter sequences are located asymmetrically relative to the center of the puff. A 1 kb probe (II) just 3' to the lacZ region but upstream of the rosy gene produces a sharp band of labeling shifted towards the distal border of the puff at 61A. Finally, hybridization using a 4.6 kb probe (III) which overlaps the rosy transcriptional unit (W. Bender, personal communication) produces labeling close to the puff border, opposite that obtained with the 5' hsp70 probe (I). These results indicate that the orientation of the insert is proximal to distal in 61A whereas the 9E insert is oriented distal to proximal. Additional in situ hybridizations using a P element probe (data not shown) indicate that the entire insert resides within the puff. The minimum size of the 61A and 9E puff domains is therefore 19 kb.

It is interesting to note that P and rosy sequences, which map outside of the region corresponding to the major heat-inducible message, are contained within the puff. If the cytological border of the puff, which we detect in this analysis by the classical acetic acid squash procedure, corresponds in position to a transitional chromosome structure in vivo, then it is significant that the puffed configuration is propagated beyond the limits of the activated transcriptional unit. The rosy gene, in the ep19.1 construction, is downstream of the heat shock gene. Thus, we cannot exclude the possibility that the rosy sequences are within the puff because the entire insert encodes an abundant but unstable primary transcript that is processed to yield the 11 kb species. We have no evidence for such a processing event and its presence would still not account for propagation of the puff domain to include the P sequences upstream of the hsp70.