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Interbands of *Drosophila melanogaster* Polytene Chromosomes Contain Matrix Association Regions

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Abstract The DNA of three previously cloned interband regions (85D9/D10, 86B4/B6, and 61C7/C8) of *Drosophila melanogaster* polytene chromosomes has been tested for the presence of matrix association regions (MAR), using the in vitro matrix-binding assay of Cockerill and Garrard. MARs were found in all three interband regions under study. These results are discussed in frames of a model postulating that interband regions of polytene chromosomes correspond to the chromosomal DNA loop borders, which can be identified in interphase nuclei using biochemical approaches. J. Cell. Biochem. 72:368–372, 1999. © 1999 Wiley-Liss, Inc.

Key words: polytene chromosomes; interbands; matrix association regions

The polytene chromosomes of *Drosophila melanogaster* are usually considered a good model to study the architecture of interphase chromosomes of higher eukaryotes. Surprisingly, it is still unclear whether pattern of bands and interbands visible in polytene chromosomes correspond to the partitioning of chromosomal DNA into loops. The latter were identified in interphase nuclei of different eukaryotic cells using both biochemical and microscopical approaches [for review, see Roberge and Gasser, 1992; Jackson et al., 1992; Razin et al., 1995; Razin, 1996]. The average size of the above loops was estimated as 25–100 kb [Jackson et al., 1990]. The loops are thought to be attached to the high salt insoluble nuclear matrix via complex DNA–protein interactions, which remain poorly characterized [Razin, 1996]. The specific DNA sequence elements known as matrix association regions (MARs) [Cockerill and Garrard, 1986] or scaffold attachment regions (SARs) [Mirkovich et al., 1984] seem to play an important role in the attachment of DNA loops to the nuclear matrix [Roberge and Gasser, 1992; Laemmli et al., 1992; Iarovaia et al., 1996]. The distribution of MARs/SARs in the genome has been intensively studied [Dijkwell and Hamlin, 1988; Phi-Van L, Stratling, 1988; Bode and Maass, 1988; Levy-Wilson and Fortier, 1989; Brun et al., 1990]. Some of the results suggest that MARs are located at the borders of functional genomic domains [Phi-Van L, Stratling, 1988; Bode and Maass, 1988; Levy-Wilson and Fortier, 1989]. It is extremely interesting to compare the distribution of MARs in the genome of *Drosophila melanogaster* with the partitioning of polytene chromosomes into morphologically distinct domains: bands and interbands. Indeed, the sizes of bands and interbands were estimated, respectively, as 5–100 kb and 0.3–4 kb [Zhimulev, 1994]. Hence, it is tempting to suggest that bands correspond to DNA loops and interbands—to the attachment regions. However, the direct analysis of the representation of MAR sequences in the interband DNA was not possible, because of the absence of an experimental approach suitable for isolation of interband DNA. The situation has changed, since Demakov and collaborators succeeded in cloning several interband regions targeted by transposon integration (Demakov et al., 1993; Schwartz et al., 1998). In the present work, we have tested three cloned interband regions for the ability to bind nuclear matrix in vitro. As follows from the obtained

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results, all these interband regions contain multiple MAR elements.

MATERIALS AND METHODS

Recombinant Clones

The recombinant clones 85–14.8, 86–7.0, and 61–3.8HB containing DNA from 85D9/D10, 86B4/B6, and 61 C7/C8 interband regions, respectively, have been described previously [Demakov et al., 1993; Schwartz et al., 1998]. Physical maps of these three clones are shown in Figure 1.

Isolation of Nuclei and Nuclear Matrices

The nuclei from *Drosophila melanogaster* larvae were isolated as described by [Shaffer et al., 1994]. Isolated nuclei were stored for ≤3 months at −20°C in a solution containing 60 mM KCl, 15 mM NaCl, 1 mM EDTA, 0.1 mM EGTA, 15 mM Tris-HCl (pH 7.4), 0.15 mM spermin, 0.5 mM spermidin, 0.5% dithiotreitol, 50% glycerol. To isolate nuclear matrices, the nuclei were washed with cold TM buffer (10 mM Tris-HCl (pH 7.4), 3 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride (PMSF)) supplemented with 0.2 mM $CuSO_4$ and resuspended in the same buffer. Then NP-40 was added (5% solution up to the final concentration of 0.1%) and suspension was incubated on ice for 10 min. This was followed by two washes with TM buffer. Permeabilized nuclei were then resuspended again in TM buffer and DNase I was added at ≤100 μg/ml. After incubation for 30 min at 37°C, an equal volume of ice-cold extraction buffer (4 M NaCl, 20 mM EDTA, 20 mM Tris-HCl, pH 7.4) was added. After incubation for 20 min at 0°C the nuclear matrices were precipitated by centrifugation for 15 min at 1,000*g* and 4°C. The pellet was washed one time by 0.5× extraction buffer and two times by TM buffer supplemented with 0.25 mM sucrose. The matrices

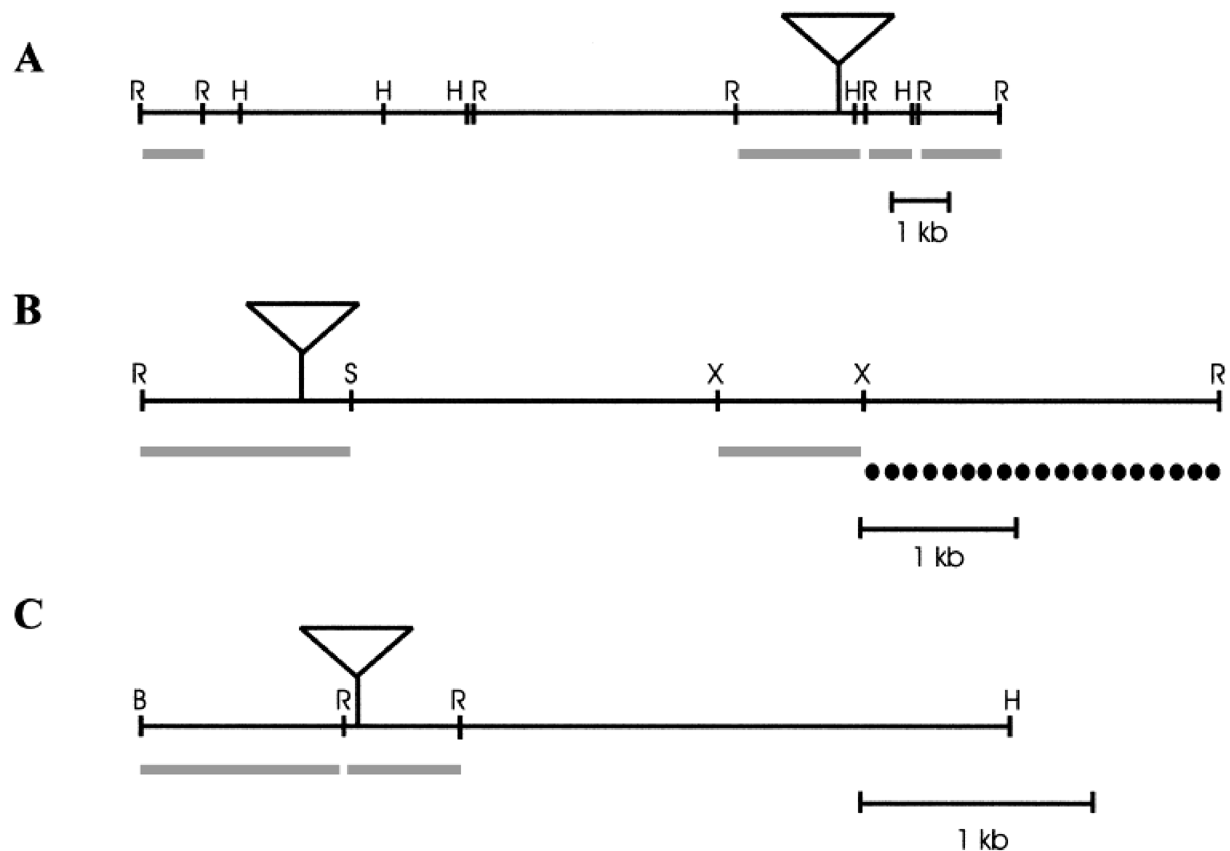

Fig. 1. Physical maps of three recombinant clones containing interband regions under study. **A:** Map of 85–14.8 clone. Gray boxes, MARs founded in the present work. The points of P-transposone insertions in the interband regions of transformed strains are marked by triangles. **B,C:** Maps of 86–7.0 and 61–3.8 HB clones correspondingly. Dotted line, *XhoI–EcoRI* fragment from 86–7.0 clone, which forms the covalent complex with the nuclear matrix proteins. R, H, X, S, B, sites of *Eco*RI, *Hin*dIII, *Xho*I, *Sal*GI, and *Bam*HI restriction endonucleases recognition. Scale bar= 1 kb, under each map.

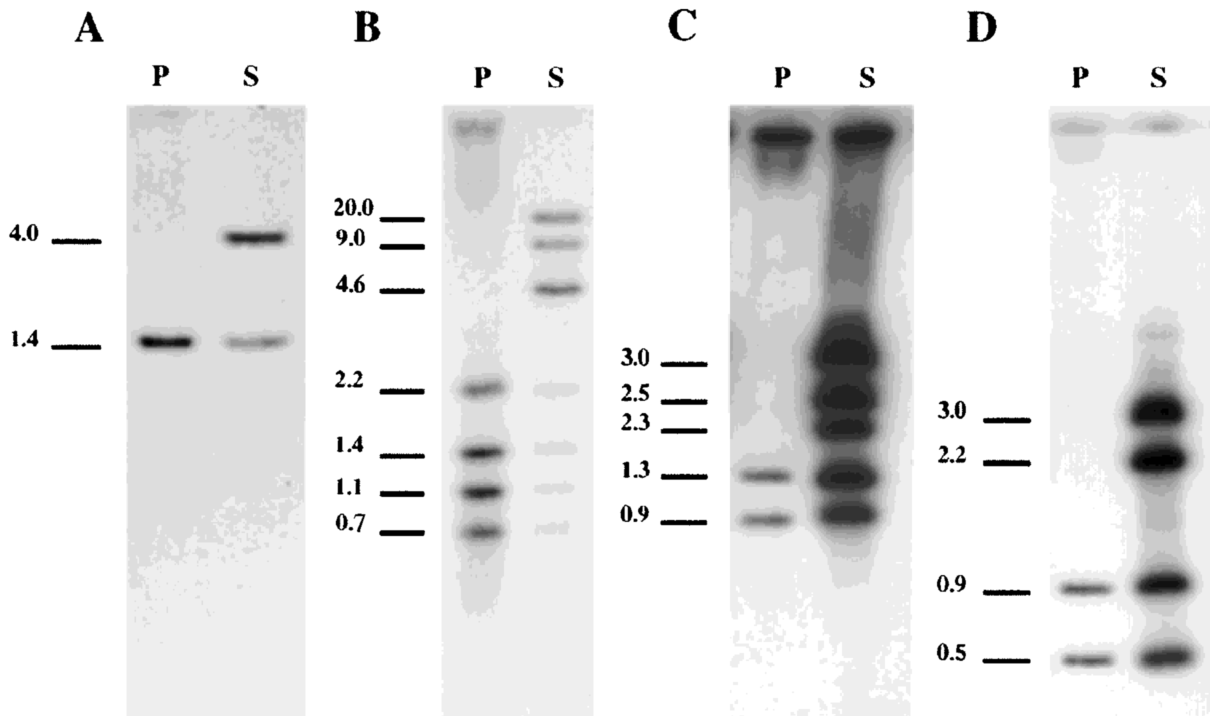

Fig. 2. Mapping of fragments binding nuclear matrix in vitro. **A:** Nuclear matrix binding of the control MAR from *Drosophila* histon gene cluster. The end-labeled fragments from the region under study were incubated with nuclear matrix; fractions of bound and unbound DNA were subsequently isolated. P (pellet)-DNA recovered from nuclear matrix fraction, i.e., MAR DNA. S-DNA recovered from supernatant, unbound DNA. **B:** Nuclear matrix binding of DNA from 85–14.8 clone digested with *Eco*RI. **C:** Nuclear matrix binding of DNA from 86–7.0 clone digested with *Eco*RI, *Sal*GI, and *Xho*I. **D:** Nuclear matrix binding of DNA from 61–3.8 HB clone digested with *Eco*RI, *Bam*HI, and *Hin*dIII. The molecular weights of the end-labeled fragments (in kilobases) are indicated at the left side of each panel.

were stored at −20°C in the above buffer supplemented with 50% glycerol.

Binding In Vitro of Cloned DNA Fragments to Nuclear Matrix

The MAR assay was carried out exactly as described by Cockerill and Garrard [1986]. The soluble and matrix bound DNA was purified by conventional procedure and analyzed using electrophoresis in 1% agarose gel. Digestion of cloned DNA by restriction enzymes and labeling of the DNA fragments were carried out as described [Maniatis et al., 1982].

RESULTS AND DISCUSSION

The sets of DNA fragments for the MAR assay were prepared by treatment of the 85–14.8 clone DNA with *Eco*RI restriction enzyme, 86–7.0 clone DNA with *Eco*RI, *Sal*GI, and *Xho*I restriction enzymes and 61–3.8HB clone DNA with *Eco*RI, *Bam*HI, and *Hind*III restriction enzymes (Fig. 1). Note that each set of restriction fragments to be tested for matrix binding contained a prokaryotic vector that served as a negative control. The bone fide MAR from the *Drosophila* histon gene cluster [Mirkovich et al., 1984; Cockerill and Garrard, 1986] was used as a positive control. The results of matrix-binding experiments are shown in Figure 2.

One can see that four fragments (2.2, 1.4, 1.1, and 0.7 kb) of the DNA of clone 85–14.8 were almost completely detained by the nuclear matrix (Fig. 2B). Three of these fragments (2.2, 1.4, and 0.7 kb map close to the site of transposon integration (Fig. 1A) and hence represent the interband region. As has been discussed previously, the border of interband cannot be mapped with >1-kb precision. Yet, according to our previous estimation, the 2-kb region containing the transposon integration site in the middle comprise for at least one-half of the corresponding interband [Demakov et al., 1993; Schwartz et al., 1998]. Among the fragments of the 86–7.0 clone, the 1.3 *Eco*RI–*Sal*GI fragment (again from the interband region; Fig. 1B) and the 0.9 *Xho*I–*Xho*I fragment have shown a

certain affinity to the nuclear matrix (Fig. 2C), although most of these fragments were recovered in the supernatant, indicating that they contain weaker MARs as compared with the MARs found in DNA of clone 85–14.8. Similarly, the 0.9-kb *Bam*HI–*Eco*RI and 0.5-kb *Eco*RI–*Eco*RI fragments, both from the 61C7/C8 interband region, as shown in Figure 1C, were found partially in association with the nuclear matrix (Fig. 2D).

It is important that prokaryotic vector (negative control) was never found in association with the nuclear matrix. By contrast, the bone fide MAR from the *Drosophila* histon gene cluster was preferentially detained by the matrix (Fig. 2A). It is of interest that one of the fragments of the 86–7.0 clone (the 2.5-kb *Xho*I–*Eco*RI fragment) apparently disappeared after incubation with the nuclear matrix, as it was not recovered from either the supernatant or the nuclear matrix. We have mentioned, however, the presence of a band with very low electrophoretic mobility in the slot containing matrix-bound fragments (Fig. 3). This may indicate that the above fragment has formed a very stable complex with the nuclear matrix proteins. Indeed, it has been described previously that some nuclear matrix proteins can form covalent complexes with MAR DNA upon incubation of this DNA with isolated nuclear matrices [Zenk et al., 1990]. The peculiarity of our case is that the complex seems to be resistant not only to sodium dodecyl sulfate (SDS) and phenol treatment, but also to treatment with proteinase K. Yet, the proteinase K-resistant nuclear matrix proteins have also been described previously [Neuter and Warner, 1985]. Formation of the complex between the 2.5-kb *Xho*I–*Eco*RI fragment of the 86–7.0 clone DNA and an unknown nuclear matrix protein can be prevented by the addition of an excessive amount of competitor prokaryotic DNA in the course of incubation of cloned DNA with the nuclear matrix. In this case, the 2.5-kb *Xho*I–*Eco*RI fragment was recovered in supernatant fraction (Fig. 3).

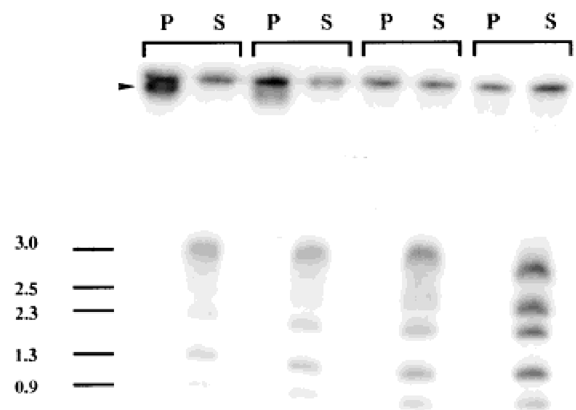

Fig. 3. Identification of fragment that forms the tight complex with nuclear matrix proteins. The nuclear matrix binding of 86–7.0 clone DNA digested with *Eco*RI, *Sal*GI, and *Xho*I was performed in the presence of different amount of competitor prokaryotic DNA. The DNA recovered both from the pellet (P) and supernatant (S) fractions was separated by electrophoresis. The concentration of competitor DNA is increasing from the left to the right. It is clearly visible that the 2.5-kb *Xho*I–*Eco*RI fragment has disappeared after incubation with the nuclear matrix, as it was not recovered from either the supernatant or the nuclear matrix (first four slots). However, notice the presence of a new band with very low electrophoretic mobility in the P slot, containing fragment covalently bound to the nuclear matrix (arrowhead). The formation of such retarding complex can be prevented by the addition of an excessive amount of competitor prokaryotic DNA (last four slots). The 1.3 kb *Eco*RI-*Sal*GI and 0.9 kb *Xho*I-*Xho*I matrix binding fragments could be seen everywhere in the P-slots after the longer exposure time. The molecular weights of end-labeled fragments (in kilobases) are indicated at the left side of the photo.

CONCLUSIONS

We may say that the present work represents a first attempt to relate the morphologically distinct organization of *Drosophila melanogaster* chromosomes with the genomic DNA partitioning into loops. The finding of MARs in all the interband regions studied strongly suggests that interbands may correspond to the DNA loop anchorage sites that were revealed in eukaryotic cell nuclei using biochemical approaches [reviewed by Roberge and Gasser, 1992; Jackson et al., 1992; Razin et al., 1995; Razin, 1996]. At the same time, we are far from the intention of proposing that all MARs are located in the interband regions. On the contrary, Mirkovich et al. [1986] have found that SARs/MARs are located in bands. As has been shown previously [Iarovaia et al., 1996], only a fraction of MARs participate in the anchorage of DNA loops on the nuclear matrix. Our current model is that exactly these MARs colocalize with interbands. The suggestion is being presently tested experimentally.

REFERENCES

Bode J, Maass K. 1988. Chromatin domain surrounding the human interferon-β gene as defined by scaffold attached regions. Biochemistry 27:4706–4711.

Brun C, Dang Q, Miassod R. 1990. Studies of an 800-kilobase DNA stretch of the *Drosophila* X chromosome: Comparing of a subclass of scaffold-attached regions with sequences able to replicate autonomously in *Saccharomyces cerevisiae*. Mol Cell Biol 10:5455–5463.

Cockerill PN, Garrard WT. 1986. Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. Cell 44:273–282.

Demakov SA, Semeshin VF, Zimulev IF. 1993. Cloning and molecular genetic analysis of *Drosophila melanogaster* interband DNA. Mol Gen Genet 238:437–443.

Dijkwel PA, Hamlin JL. 1988. Matrix attachment regions are positioned near replication initiation sites, genes, and an interamplicon junction in the amplified dihydrofolate reductase domain of Chinese hamster ovary cells. Mol Cell Biol 8:5398–53409.

Iarovaia OV, Hancock R, Lagarkova MA, Miassod R, Razin SV. 1996. Mapping of genomic DNA loop organization in a 500-kilobase region of the *Drosophila* X chromosome using the topoisomerase II-mediated DNA loop excision protocol. Mol Cell Biol 16:302–308.

Jackson DA, Dickinson P, Cook, PR. 1990. The size of chromatin loops in HeLa cells. EMBO J 9:567–571.

Jackson DA, Dolle A, Robertson G, Cook PR. 1992. The attachments of chromatin loops to the nucleoskeleton. Cell Biol Int Rep 16:687–696.

Laemmli UK, Kas E, Poljak L, Adachi Y. 1992. Scaffold-associated regions: cis-acting determinants of chromatin structural loops and functional domains. Curr Opin Genet Dev 2:275–285.

Levy-Wilson B, Fortier C. 1989. The limits of the DNase I-sensitive domain in the human apolipoprotein B gene coincide with the locations of chromosomal anchorage loops and define the 5′ and 3′ boundaries of the gene. J Biol Chem 264:21196–21204.

Maniatis T, Fritsch EE, Sambrook J. 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Mirkovich J, Mirault E, Laemmli UK. 1984. Organization of the higher order chromatin loop: Specific DNA attachment sites on nuclear scaffold. Cell 39:323–332.

Mirkovich J, Spierer P, Laemmli UK. 1986. Geens and loops in 320 000 base pairs of the Drosophila melanogaster chromosome. J Mol Biol 190: 255–258.

Neuer B, Werner D. 1985. Screening of isolated DNA for sequences released from anchorage sites in the nuclear matrix. J Mol Biol 181:15–25.

Phi-Van L, Stratling WH. 1988. The matrix attachment regions of the chicken lysozyme gene co-map with the boundaries of the chromatin domain. EMBO J 7:655–664.

Razin SV. 1996. Functional architecture of chromosomal DNA domains. Crit Rev Eukaryot Genes Expr 6:247–269.

Razin SV, Gromova II, Iarovaia OV. 1995. Specificity and functional significance of DNA interaction with the nuclear matrix: New approaches to clarify the old questions. Int Rev Cytol 162B:405–448.

Roberge M, Gasser SM. 1992. DNA loops: Structural and functional properties of scaffold-attached regions. Mol Microbiol 6:419–423.

Shaffer CD, Wuller JM, Elgin SCR. 1994. Preparation of *Drosophila* nuclei. Methods Cell Biol 44:185–189.

Schwartz YuB, Demakov SA, Zhimulev IF. 1998. Cloning and analysis of DNA from *Drosophila melanogaster* polytene chromosomes interband regions 85D9/D10 and 86B4/B6. Genetika (Moscow) 34:1081–1089.

Zenk DW, Ginder GD, Brotherton TW. 1990. A nuclear matrix protein binds tightly to DNA in the avian beta-globin gene enhancer. Biochemistry 29:5221–5226.

Zhimulev IF. 1994. Morphology and structure of polytene chromosomes. Adv Genet 34:1–497.