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Sequence of scs and scs′ *Drosophila* DNA fragments with boundary function in the control of gene expression

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Submitted March 5, 1992 EMBL accession nos X63731 and X63732

Boundary structures are essential chromatin formations which can behave as insulators when bracketing a gene. This means that they behave as functional barriers when inserted between an enhancer and a test gene, in this way insulating the gene from the effect of enhancers located outside the boundaries, while maintaining the activity of the enhancers within the boundaries (1). Boundary structures have been characterized genetically in the *Drosophila* Bithorax complex (2), and functionally and structurally in the chicken lysozyme gene (3) and in the hsp 70 heat shock genes of *Drosophila* (4−6). We report here the sequence of the scs and scs′ DNA fragments from the proximal and distal ends of the 87A7 heat shock region of *Drosophila melanogaster* which carry specific chromatin structures (scs) with boundary function (5). In functional tests involving transgenic *Drosophila* strains carrying a reporter gene, a promoter, an enhancer and the scs elements in different arrangements, these fragments fulfilled the above-mentioned criteria of the boundary function, i.e. the expression of the reporter gene was abolished when the scs or the scs′ fragment was inserted between the enhancer and the promoter, while scs elements placed upstream of the enhancer did not influence the expression of the reporter gene (5). It was also demonstrated that, due to the insulating properties of the boundary structures, a reporter gene bracketed by scs elements showed position independent expression in transgenic animals (6).

The position of the specific chromatin structures was mapped by the indirect end labeling hybridization technique, using appropriate marker fragments (4). In this way the scs region was localized on a 1.8 kb BglII−BamHI fragment which is 4.3 kb from the 3′ end of the proximal hsp 70 gene copy toward the centromere (4, 5), while the scs′ region is on a 1.1 kb EcoRI−PstI fragment located 1.96 kb from the 3′ end of the distal hsp 70 gene copy toward the telomere (4, 5). These fragments were cloned by genomic walking, using the proximal and distal terminal fragments of plasmid 122 (7), which carries the hsp 70 heat shock genes from the 87A7 cytogenetic region, and sequenced by the dideoxy chain termination method. The numbering starts at the BglII site in the scs fragment and at the EcoRI site in the scs′ fragment.

The specific chromatin structures at the boundaries of the heat shock region consist of two strong nuclease hypersensitive sites separated by a nuclease resistant core. The position of the nuclease hypersensitive blocks was mapped on 45 cm long agarose gels (4). The accuracy of this mapping is about 50 bp. On the basis on these mapping data, the nuclease hypersensitive blocks are between positions 870−1080 and 1370−1530 in the scs fragment and between positions 50−160 and 370−490 in the scs′ fragment. The DNA segments corresponding to the nuclease resistant core are very AT rich (73.2% in scs and 73.4% in scs′). Although no extensive sequence homology was found between the two fragments, there were several short homologous blocks. Due to the high AT content of the sequences, however, the relevance of these homologies as recognition sequences for regulatory proteins can not be deduced from the sequence data.

REFERENCES

1. Eissenberg,J.C. and Elgin,S.C.R. (1991) *Trends Genet.* **7**, 335−340.
2. Gyurkovics,H., Gausz,J., Kummer,J. and Karch,F. (1990) *EMBO J.* **9**, 2579−2585.
3. Bonifer,C., Vidfal,M., Grosveld,F. and Sippel,A.E. (1990) *EMBO J.* **9**, 2843−2848.
4. Udvardy,A., Maine,E. and Schedl,P. (1985) *J. Mol. Biol.* **285**, 341−358.
5. Kellum,R. PhD Thesis. Princeton University. 1990.
6. Kellum,R. and Schedl,P. (1991) *Cell* **64**, 941−950.
7. Goldschmidt-Clermont,M. (1980) *Nucleic Acids Res.* **8**, 235−252.

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