Relation of Chromosome Structure and Gene Expression

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Relation of chromosome structure and gene expression

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[Plate 1]

We have been able to map specific DNA fragments at the bases of chromatin loops with the help of a novel extraction procedure by using lithium-3′,5′-diiodosalicylate. One such scaffold-attached region (SAR) is found in the non-transcribed spacer in each repeat of the histone gene cluster, on a 657 base pair (b.p.) restriction fragment. Exonuclease III digestion has localized two protein-binding domains on the SAR of the histone cluster. Each covers approximately 200 b.p. and they are separated by a nuclease-accessible region of about 100 b.p. These domains are rich in sequences closely related to the topoisomerase II cleavage consensus. We have studied the scaffold association of three developmentally regulated genes of *Drosophila melanogaster*: alcohol dehydrogenase (*Adh*), the homoeotic gene fushi tarazu (*ftz*) and *Sgs-4*, a gene encoding one of the glue proteins secreted by third-instar larvae. We find regions attached to the nuclear scaffold (SARS) both 5′ and 3′ of all three genes, defining small domains ranging from 4.5 to 13 kilobases. In the case of *Adh*, a gene with two promoters, we find two upstream and two downstream SARS. Those 5′ of the gene co-map with regulatory regions for the adult and the larval transcripts, respectively. For *Sgs-4*, the 5′ SAR covers 866 b.p. immediately upstream of the transcript, and encompasses the 200 b.p. regulatory region defined by two deletion mutants that produce little or no Sgs-4 protein. In *ftz* the 5′ SAR is found 4.8 kilobases upstream of the start of transcription within a 2.5 kilobase element required for a high level of *ftz* expression in the early embryo. Sequence analysis of five upstream SARS reveals clusters of sequences closely related to the cleavage consensus of topoisomerase II. In addition, they contain multiple copies of two sequence motifs: a specific 10 b.p. A-rich sequence, and another 10 b.p. T-rich stretch.

In conclusion, the intimate association of the SAR with the upstream/enhancer elements, the presence of clustered sequences highly homologous to the topoisomerase II cleavage consensus, and the localization of topoisomerase II in the scaffold, suggest a structure–function relation between chromosome organization and gene expression.

CHROMOSOMAL SUBUNITS: NUCLEOSOMES AND LOOP

The nucleosome subunit, composed of the core histones, and histone H1 determine the structure of the 30 nm basic chromatin fibre observed in the eukaryotic nucleus. The packaging of the DNA molecule into nucleosome subunits and the solenoidal arrangement of these into the 30 nm fibre compresses the length of the DNA approximately 30- to 40-fold (Widom & Klug 1985; Felsenfeld & McGhee 1986). The nucleosomes alone do not determine the folding pattern of the chromatin fibre in the compact chromosomes, where the packaging ratio is approximately 10000-fold. Various models for this higher-order folding have been proposed; of these the loop model has substantial experimental support from electron microscopy,

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sedimentation and nuclease digestion studies (reviewed by Paulson (1986)). Evidence for chromatin loops was initially reported by Cook & Brazell (1976), Benyajati & Worcel (1976), Igo-Kemenes & Zachau (1977) and Paulson & Laemmli (1977).

In this model the fibre is first folded into loops consisting of about 30–100 kilobases (kb) of DNA, and the loops are fastened at their bases by non-histone proteins (Laemmli *et al.* 1978). In the condensed chromosome, neighbouring loops are somehow held together by protein–protein and protein–DNA interactions that form an internal network or scaffolding along the chromosomal axis. The role of the histones in this model is to package the DNA of each loop, whereas the scaffolding proteins organize the bases of loops. It is not known how neighbouring loops are arranged with respect to one another, but given the unineme concept of chromosome structure, a helical arrangement progressing along the axis of the chromatid is conceivable (Marsden & Laemmli 1979; Adolph 1980, 1981; Rattner & Lin 1985).

It is attractive to think of chromatin loops as the higher-order structural subunit of chromosomes, not as a strictly conserved, regularly repeated structure like the subunit protein of a virus, but as subunits with certain biochemical and structural features in common, over which modifications can be applied. Thus one would expect to find different classes of loops defining different chromosomal regions, just as modifications of nucleosomes could define different domains. Interaction of the bases of the chromatin loops with the scaffolding network or with other subnuclear elements would maintain order in the nucleus, facilitating gene expression, chromatin templating, chromosome segregation and orderly replication. The dynamic changes of chromosomes (condensation, decondensation, puffing, etc.) could be driven by dynamic changes in the scaffold that would drag along the associated chromatin loops, rather than by a synchronized modification of all the nucleosomes within a given loop. In the following sections we briefly review current concepts of the structure and function of the chromatin loop.

THE MAJOR PROTEIN OF THE METAPHASE SCAFFOLD, Sc1, IS TOPOISOMERASE II

The microscopic observation of looped structures in metaphase chromosomes and interphase nuclei after the extraction of histones suggested that some of the proteins left in the extracted chromosomes or nuclei function as fasteners to constrain the DNA into looped domains. These residual structures were variously called the chromosomal scaffold, nuclear matrix or scaffold. Because harsh procedures were initially used to remove the histones, it was difficult to rule out the possibility that the observed organization was artefactual. Recent data, however, have provided strong evidence that the scaffold structure of chromosomes and nuclei is not artefactual and is likely to be of biological importance. Attempts to identify the minimal set of proteins necessary to restrain DNA loops in metaphase chromosomes led to the identification of two proteins, Sc1 and Sc2 (170 and 135 kDa) (Lewis & Laemmli 1982). The Sc1 protein is the most abundant non-histone protein found in metaphase chromosomes; it binds DNA and is present in approximately three copies per average DNA loop in human metaphase chromosomes. This number is consistent with the postulated role of these proteins as 'loop-fasteners'. With the help of a specific antiserum raised against Sc1, this protein has been shown to be identical to topoisomerase II (Earnshaw & Heck 1985; Earnshaw *et al.* 1985; Gasser *et al.* 1986). Topoisomerase II has also been shown to be a major component of the residual nuclear matrix of *Drosophila* embryonic cells (Berrios *et al.* 1985). Immunolocalization of

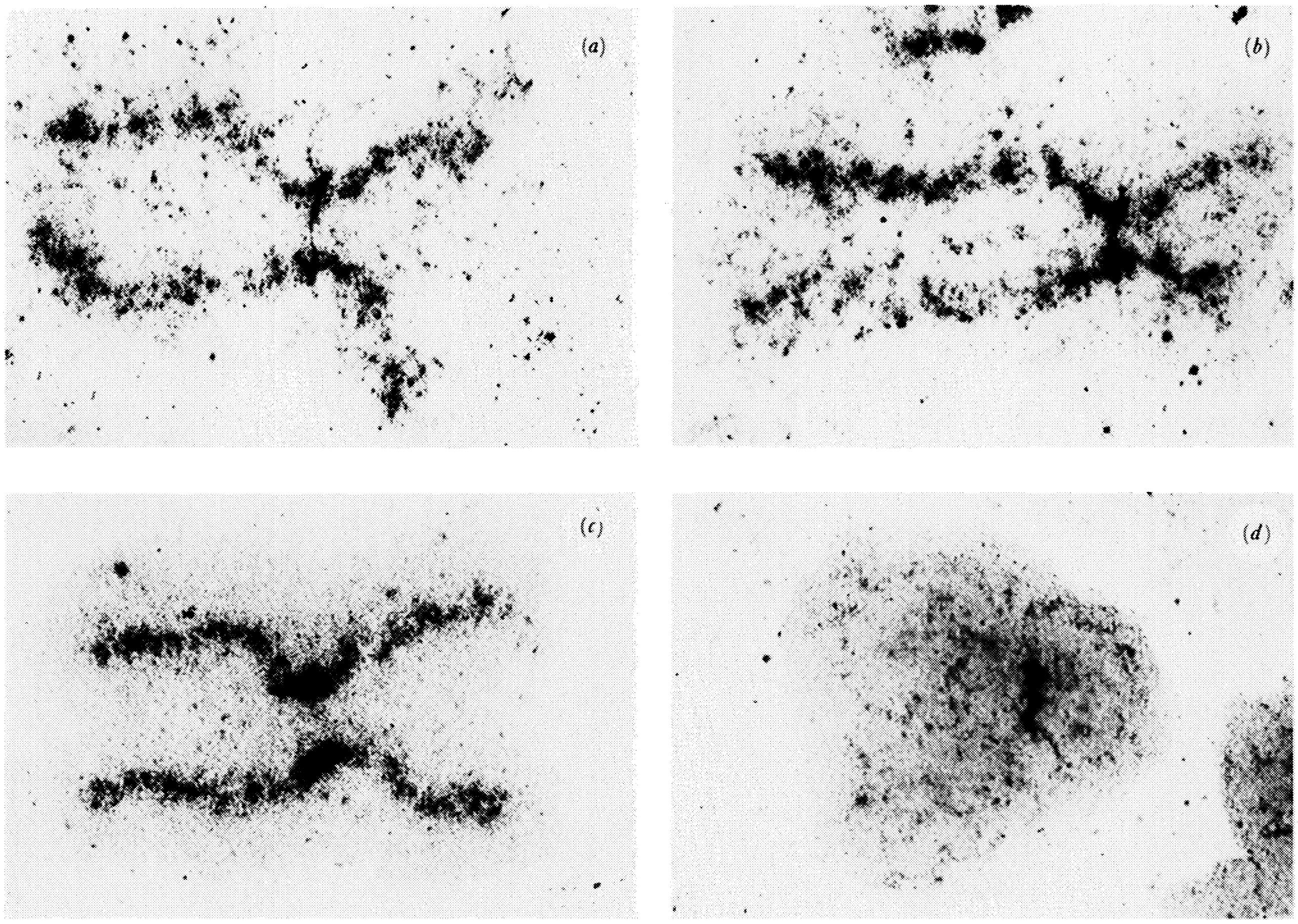

FIGURE 1. Immunolocalization of topoisomerase II in metaphase chromosomes. In (*a*–*c*) the antibody directed against topoisomerase II (Sc1 protein, molecular mass 170000 Da) identifies an axial element that extends the length of the chromatid going through the kinetochore elements. Some of these micrographs provide evidence of the substructural organization of the scaffold, which appears to consist of an assembly of foci, forming in places a zig-zag or coiled arrangement. A peroxidase-coupled secondary antibody was used for the staining reaction (Gasser *et al.* 1986) with both immune (*a*–*c*) and preimmune (*d*) sera. The kinetochore elements, but not the axial staining, are seen in the control (*d*) (Gasser *et al.* (1986), see also Earnshaw & Heck (1985)).

topoisomerase II, both by immunofluorescence and electron microscopy, allowed identification of the scaffold structure directly in 'native', gently expanded chromosomes. The immunopositive reaction is found along a central, axial region, extending through the kinetochore along the entire length of the chromatid. In histone-depleted chromosomes, where the scaffold is further expanded, the scaffold appears to be an assembly of foci that in places forms a zig-zag arrangement (Gasser *et al.* 1986; Earnshaw & Heck 1985) (see figure 1, plate 1). These foci are more closely packed in the compact, histone-containing chromosome, and some micrographs suggest a helical progression along the chromatid. It is tempting to propose that each focus represents a scaffold 'subunit', consisting of an assembly of bases of loops.

These data establish the existence of an axial scaffolding structure in unextracted metaphase chromosomes; they confirm that the protein Sc1 is indeed a component of the scaffold, and more importantly, they suggest a structural as well as an enzymic role for topoisomerase II. The nuclear matrix of interphase nuclei is obtained under conditions similar to that for the metaphase scaffold, but is much more complex in morphology and composition. It is composed of the peripheral lamina, an ill-defined internal network and a residual nucleolus (reviewed by Nelson *et al.* (1986)). The peripheral lamina is by far the best-characterized component of the nuclear matrix (reviewed by Gerace (1986)). Chromatin appears closely associated with the peripheral lamina and the lamina proteins bind DNA *in vitro*. It has been proposed that this peripheral structure may serve to organize one level of organization of the chromatin loops by serving as an attachment structure for the chromatin fibre. An experimentally distinguishable, additional level of organization appears to be due to the attachment of the chromatin fibre to the ill-defined internal network (Lebkowski & Laemmli 1982*a*, *b*). Topoisomerase II is an important component of the internal network of nuclear matrices; immunolocalization of topoisomerase II in nuclei reveals a diffuse general staining of the interior lumen excluding the nucleolus (Berrios *et al.* 1985).

Specific scaffold-associated DNA regions (sars)

As a test for the loop model one might hope to find specific regions spaced along the DNA at which the scaffold interaction occurs. Such specific scaffold-associated regions (SAR) have been identified with the help of a novel extraction procedure that uses lithium-3′,5′-diiodosalicylate (LIS). At low concentrations and in 'physiological' salt buffers, this compound extracts histones and other proteins under conditions apparently 'mild' enough to preserve a specific scaffold–DNA interaction. The LIS is removed by repeated washing and the extracted nuclei are digested to completion with various restriction enzymes. A restriction fragment that contains an SAR cosediments with the nuclear scaffold. In the *Drosophila* system we have mapped 18 SARS near a variety of genes that are transcribed by polymerase II (Gasser & Laemmli 1986*a*, *b*; Mirkovitch *et al.* 1984, 1986), extending over 400 kilobase pairs of DNA, 320 of which are within a chromosomal 'walk' around the *rosy* locus (see figure 4). A number of experiments have been done to determine the biochemical nature and the functional significance of scaffold–DNA binding.

At present, our basic observations are as follows: (1) SARS can be mapped to fragments ranging from 0.6 to 1 kilobase pairs in size, containing multiple sites of scaffold–DNA interaction; (2) in *Drosophila* SARS are found in non-transcribed regions. For the mouse kappa light-chain gene, however, an SAR was identified adjacent to the enhancer sequence in a transcribed region (Cockerill & Garrard 1986); (3) the distance between two adjacent SARs varies between 4.5 and 112 kilobase pairs; (4) one or several differentially regulated genes can occur between two SARs; (5) in *Drosophila* several enhancer-like elements for developmentally regulated genes cohabit with SARs; (6) no changes have been observed in scaffold attachment upon the induction of transcription; (7) the SAR interactions are similar in nuclei derived from developmentally different cells; (8) scaffolds obtained from metaphase chromosome clusters bind the same SAR sequences as the nuclear scaffold. These observations are discussed in more detail in the following sections.

SARS CONTAIN CLUSTERS OF THE TOPOISOMERASE II CONSENSUS SEQUENCE AND TWO ADDITIONAL SEQUENCE MOTIFS

The best-characterized SAR is found on a 657 b.p. fragment in the non-transcribed spacer between the H1 and H3 genes. This attachment is observed in all the tandemly organized histone repeats, defining small 5 kilobase pair loops (figure 2). Exonuclease III digestion studies have identified two protein binding domains within this SAR, each covering about 200 b.p., as depicted in figure 2. Studies of other SAR fragments also reveal multiple binding sites within rather large regions (up to 1.1 kilobase pairs). Each individual binding domain is able to mediate scaffold association, albeit with a sometimes reduced affinity (Gasser & Laemmli 1986*a*).

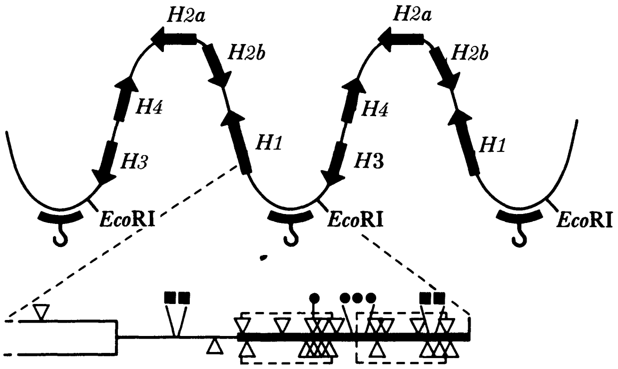

FIGURE 2. Repeat of histone genes: one repeat, one loop. Two repeats of the 5 kb DNA fragment that contains all five *Drosophila* histone genes are shown in the upper part of the figure. The 657 b.p. scaffold-attached region (SAR) occurs in the non-transcribed spacer between the *H1* and *H3* genes and is indicated by a bar containing a hook. Approximately 100 tandem histone gene repeats are present in the genome, forming a series of small loops. Sequence motifs common to a number of SARs are indicated in the enlarged map of the SAR. ▽, Sequences with 70% homology to the topoisomerase II consensus sequence (Sanders & Hsieh 1985), in either the Watson (top) or Crick (bottom) strand. ●, The 10 b.p. A-box; ■, the 10 b.p. T-box described in the text. Two 200 b.p. domains within the SAR (here encompassed by dotted lines) were resistant to exonuclease III digestion in intact scaffolds, indicating the presence of protein–DNA complexes (Gasser & Laemmli 1986*a*, *b*).

The presence of topoisomerase II in the metaphase scaffold prompted us to screen the available SAR sequences for the *Drosophila* topoisomerase II consensus sequence (Sander & Hsieh 1985). All SARs tested contain a strikingly large number (from 8 to 17 per fragment) of sequences related to the 15 b.p. topoisomerase II cleavage site (GTN(A/T)A(T/C) ATTNATNN(G/A)) (Gasser & Laemmli 1986*a*, *b*). Although this is a weak and loosely defined consensus, two results suggest that the clustering of such sequences within the SAR fragments is significant. First, Udvardy *et al.* (1985) have shown that the SARS of the histone cluster and of the heat-shock protein 70 heat-shock genes are major targets for topoisomerase II cleavage *in vitro*. Secondly, the DNA regions that are not bound to the scaffold generally do not contain such clusters of topo II boxes. However, the occurrence of the consensus alone does not appear to be sufficient to create an SAR, because in the *Adh* gene region we found a fragment with several topo II boxes which was not scaffold bound (Gasser & Laemmli 1986*b*). *In vivo* localization of drug-induced DNA topoisomerase II cleavage sites in the heat-shock protein 70 genes has revealed multiple specific cleavage sites both at the 3′ and 5′ ends of the genes. Only minor cutting was observed in the 5′SAR region of this gene, although an enhanced cleavage was observed in the SAR after heat-shock activation (Rowe *et al.* 1986). Thus the presence of the topo II boxes in the SAR may represent only potential sites of action for a topoisomerase II *in vivo*. The SARS that have been analysed contain two additional 10 b.p. sequence motifs, the T box (TT(A/T)T(T/A)TT(T/A)TT) and the A-box (AATAAA(T/C)AAA) (figure 2). The pattern of topo II, T- and A-boxes of four different SARS are shown in figures 2 and 3. The clustering of topo II boxes in the various SARS is impressive, but does not appear to follow a simple pattern. Note, however, that the T-box is often found downstream of the A-box: this run of thymidine residues may be responsible for bends and kinks in the DNA or may exclude nucleosome formation.

SARS ARE OFTEN CLOSE TO PROMOTERS AND COHABIT WITH UPSTREAM REGULATORY SEQUENCES

For three developmentally regulated genes of *Drosophila* (*Adh*, *Sgs-4* and *ftz*) the SARS 5′ of the genes have been found to cohabit with regulatory sequences up to 4.5 kilobase pairs upstream of the start of transcription (see figure 3) (Gasser & Laemmli 1986*b*; Hiromi *et al.* 1985; McNabb & Beckendorf 1986; Posakony *et al.* 1985). Remarkably, for the *Adh* locus, from which two transcripts are made, two upstream/enhancer-like regulatory regions were identified as well as two 5′ scaffold-attached regions. The sequences required for tissue-specific expression of *Adh* and *ftz*, on the other hand, are not scaffold-bound, nor are the actual coding sequences for any of the genes studied. For each of these three highly expressed, developmentally regulated loci, SARS are also found 3′ of the transcription units. These could interact with the 5′ SARS to form small loops ranging in size from 4.5 to 13 kilobase pairs, each containing one gene.

The term ‘cohabitation’ describes our finding that the restriction enzyme fragments defining upstream/enhancer-like elements of these genes also contain SARS. Neither type of mapping data excludes the possibility that the two DNA elements might still be separable by the appropriate experimentation. In the mouse immunoglobulin kappa gene, the matrix-binding site is adjacent to, and separable from, the enhancer sequence (Cockerill & Garrard 1986). Yet for the *Drosophila Sgs-4* gene, this cohabitation appears quite intimate; a 710 b.p. region immediately upstream from the transcription start site contains the essential regulatory sequences, the SAR, and the DNase I hypersensitive sites associated with gene activity (McNabb & Beckendorf 1986). Such a close functional link between the SAR and upstream regulatory element is not observed in the major heat-shock gene *hsp 70*. This SAR is upstream of the DNase I hypersensitive sites associated with active transcription, and is also upstream of control

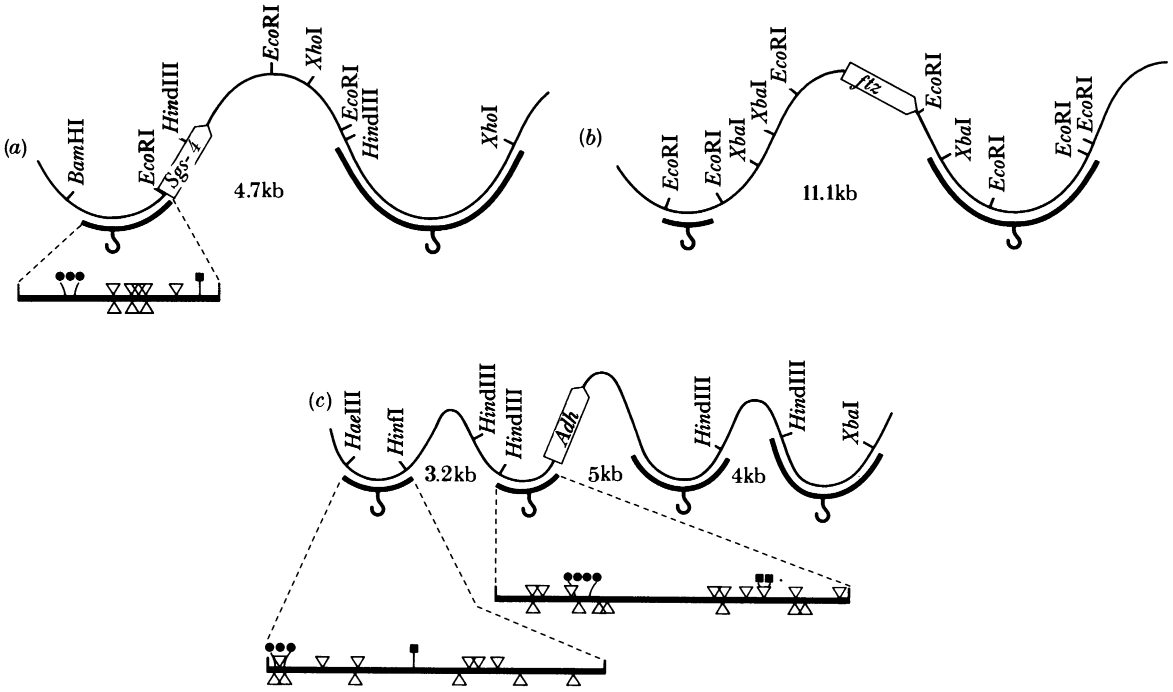

FIGURE 3. Cohabitation of SARS with upstream–enhancer elements. (*a–c*) The loop structure (Gasser & Laemmli 1986*a*) for three developmentally regulated genes: the glue protein gene, *Sgs-4* (McNabb & Beckendorf 1986), the homoeobox-containing gene fushi tarazu, *ftz* (Hiromi *et al.* 1985) and the alcohol dehydrogenase gene, *Adh* (Posakony *et al.* 1985), respectively. SARS are found both 5′ and 3′ from the coding sequences (open boxes) as indicated by the hooked bars. As in the case of the histone gene cluster, the loop sizes of these temporally and highly expressed genes are small, at maximum 11 kilobases (kb). In the case of *Adh*, which has two promoters and two transcripts from the same coding unit, there are two upstream and two downstream SARS. For all three genes the 5′ SARS cohabit with enhancer-like regulatory elements (Gasser & Laemmli 1986*b*). Loop size measured from centre to centre of two adjacent SARS is given below the corresponding loops. The presence of several sequence motifs is shown in the enlarged maps of the SARS for *Sgs-4* and *Adh*. ▽ represents the topoisomerase II consensus, ● the A-box and ■ the T-box as discussed in figure 1 (see the text). For orientation a selection of relevant restriction sites are given.

elements that are necessary and apparently sufficient for its complete regulation (Dudler & Travers 1984; Udvardy & Schedl 1984). The observation that the SAR of the *hsp 70* gene lacks the usual series of upstream A-boxes suggests to us that different SAR types may correlate with differently regulated genes. For example, a developmentally regulated gene may have a ‘regulated’ SAR fragment containing both topo II clusters and A-boxes, whereas a household-type gene may have a ‘constitutive’ SAR without A-boxes. If A-boxes are diagnostic of ‘regulated’ SARS, then one would predict that the histone SAR is involved in transcriptional control.

What is the importance of the loop size or the proximity of the 5′ SAR or both? The data available at present fit best with the notion that in *Drosophila* the highly expressed genes are found in small loops of 4–17 kilobase pairs whereas genes with less abundant transcriptional activity are found in much larger loops of 50 kilobases or more. Examples of the former class would be *hsp 70, actin 5C*, the histone cluster, *Adh, Sgs-4* and *ftz*, and for the latter class, all the genes localized within the 320 kilobases ‘walk’ around the *rosy* locus (figure 4). These observations suggest an inverse correlation between the potential level of transcription and loop size.

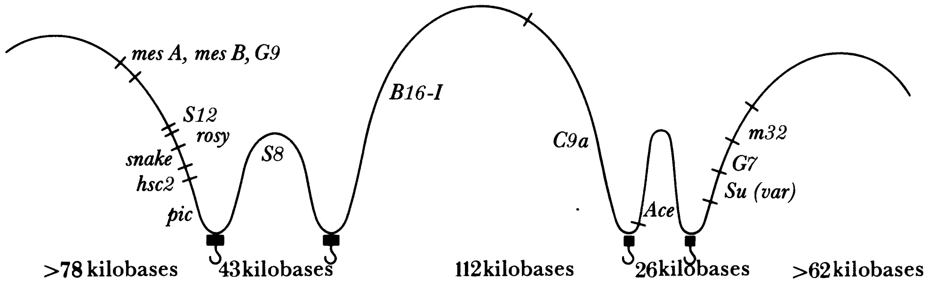

FIGURE 4. Loops and genes in 320 kb of *Drosophila* DNA. The loop organization is shown in the diagram for a 320 kb region surrounding the *rosy* and *Ace* loci from *Drosophila* Kc cells (Mirkovitch *et al.* 1986). The various genetic loci are indicated by lines and the position of the SARS by hooked bars. The loop sizes in this region are large as indicated in the figure and the various transcripts are of low abundance, an observation that suggests an inverse correlation between the potential level of transcription and loop size.

DIFFERENT SAR FAMILIES EXIST

We have previously identified a family of SAR fragments related by hybridization to the scaffold-associated region of the *hsp 70* genes (Mirkovitch *et al.* 1984). This SAR contains AT-rich sequence motifs (called X) which are repeated at about 40 cytogenetic loci in the genome of *D. melanogaster* (Lis *et al.* 1981). By hybridization with an *hsp 70* SAR fragment, we found that most of the DNA fragments identified by this probe are also associated with the nuclear scaffold. This procedure identifies a 'heat-shock' SAR family.

More recently, *in vitro* binding and competition experiments identify other families. We have shown that addition of cloned, exogenous SAR fragments bind to the nuclear scaffolds in the presence of total endogenous, genomic DNA (appropriately digested). The binding of the exogenous SAR DNA occurs with a specificity indistinguishable from that of the endogenous, genomic DNA. We have tested the possibility that addition of increasing amounts of DNA that contains SARS to the nuclear scaffold leads to a displacement of the genomic homologue and possibly other SARS. Such competition experiments show that exogenously added SARS can effectively compete for the endogenous homologue and all the other SARS we have tested, albeit with different effectiveness. Pairwise competition experiments identify different families, a class being defined as a poor competitor for certain SARS but an effective competitor for others. As an example, the SAR of the actin *5C* gene competes effectively against the two upstream SARS of *Adh* but less well against the SARS of the histone, *hsp 70* and the fushi tarazu (*ftz*) genes (Mirkovitch, in preparation). The significance of these different SAR families is not known, but they may represent different chromosomal subcompartments; a given SAR family could bring dispersed genes into a common nuclear microenvironment permitting the sharing of common needs.

ARE SAR POSITIONS AND LOOP STRUCTURE TISSUE-SPECIFIC?

One can imagine that certain attachment sites are preferentially lost or created during cell differentiation, perhaps mediated by specific transcription factors, allowing for the modulation of nuclear domains as required by the cell's specific pattern of gene expression. It follows that some SARS, namely those near housekeeping genes, would remain constant in all proliferating cells.

In both the mouse and *Drosophila* systems, few significant differences in loop organization have been seen among different cell types (Gasser & Laemmli 1986*a*, *b*; Cockerill & Garrard 1986). This may be due, however, to the character of the assay for these sites. Because SARs are detected after extraction of the histones, any genomic fragment with the potential to bind might be detected as scaffold-bound. Thus it is possible that some fragments identified as an SAR in our assay *in vitro* may actually have their scaffold binding sites blocked by histones or other proteins in the intact nucleus (Gasser & Laemmli 1986*b*).

SOME SARS COMAP WITH BOUNDARIES OF 'ACTIVE' DOMAINS

Non-transcribed regions of chromatin at the borders of actively transcribed regions are often associated with highly ordered or 'static' nucleosome organization, whereas a more 'blurred' organization of nucleosomes is seen in the nearby transcribed regions. In two cases, SARs co-map with such regions of 'static' nucleosome organization bordering actively transcribed domains. One is the non-transcribed region of the H1–H3 spacer, in which the two protein-binding elements of the SAR coincide quite well with nucleosome-sized particles detected by micrococcal nuclease digestion (Worcel *et al.* 1983). In addition, the SARs located 5′ of the *hsp 70* genes (at both loci *87C1* and *87A7*) fall into regions of static, 'phased' nucleosomes (Udvardy & Schedl 1984). Like the SAR interaction, the static pattern is maintained during transcriptional activation of these genes. These data suggest that some SARs may be involved in defining boundaries.

SARS MAY DEFINE A POSITIONALLY INDEPENDENT FUNCTIONING UNIT

Do SARs define a region that will function independently of its position in the genome? The available data from P-element transformation with the *ftz* gene are consistent with this notion. Constructs containing either both the 5′ and the 3′ SARs of the *ftz* gene, or just the gene with 5′ SAR were used; when both SARs were present in the transforming vector none of the eight *ftz* transformants showed a position effect. Among the seven transformants obtained with the construct lacking the 3′ SAR, four showed low levels of expression, presumably due to the site of insertion (position effect). Another example concerns the expression of the *Sgs-4* glue-protein gene. Individual transformed flies derived from constructs that include the 5′ regulatory SAR region, but not the 3′ SAR, express the introduced *Sgs-4* gene with great variability, and no puffing was observed at the various sites of insertion.

Both the *Sgs-4* and the *ftz* genes are highly transcribed genes with closely positioned SARs. One might expect that transformation with genes transcribed at low levels, which have no nearby SAR, would be less susceptible to position effects. Indeed, this is the case for the weakly expressed *rosy* gene (Spradling & Rubin 1983), a gene for which no closely positioned SAR was found.

CONCLUSION AND SPECULATION

The genome is organized into loops that appear to have both a structural and functional role. The SAR sequences are proposed to define the boundaries of these loops. These scaffold-attachment regions are proposed to have primarily a structural role to maintain order in the nucleus and metaphase chromosomes and to define the various subchromosomal regions that can be observed in nuclei and chromosomes. Examples of such subchromosomal regions are the heterochromatic regions, the early and late replicating chromosomal bands (Goldman *et al.* 1985) and the so-called D-Bands which contain active chromatin (Kerem *et al.* 1984). Different SAR families exist and it is tempting to speculate that the different subchromosomal regions are each defined by a different SAR family. Topoisomerase II appears to be a pivotal protein in chromosomal structure and nuclear order. It is present in amounts sufficient to serve as a 'loop-fastener', and has been shown to be a major component of the chromosomal scaffold. The activity of this enzyme is likely to be highly regulated and may be involved in the release of stress during transcription or replication, as well as in the decatenation of replicated DNA (Uemura & Yanagida 1984; Holm *et al.* 1985). If topoisomerase II exerts its action at SAR sequences at the bases of loops, it would be strategically located to control long-range order in chromatin domains during these processes. SARS may also serve as preferred sites at which DNA replication begins or ends.

In *Drosophila*, loops may range in size from 5 to 112 kilobase pairs and SARS tend to be found close to the promoter elements of potential highly active genes. The SARS of three developmentally regulated genes (*Adh, Sgs-4* and *ftz*) cohabit with the upstream/enhancer-like elements. The scaffold interaction may serve to bring distant regulatory sequences close together to create functional complexes for the regulation of transcription, by analogy to the mini-loops discussed by Ptashne (1986). Genes with less abundant transcriptional activity are found in larger loops and we find a loose inverse relation between loop size and the level of transcription of genes contained within the loop.

Seen on a larger scale, the positioning of SARS close to active genes may serve to bring together those sequences that need to be acted upon by specific regulatory proteins or polymerases. DNA–protein interaction studies in prokaryotic systems have taught us that diffusion-controlled, bimolecular processes are much too slow to account for the measured rates by which specific DNA binding proteins find their target site. Therefore two diffusion-facilitating mechanisms have been proposed to account for the enhanced rate at which specific DNA–protein association proceeds (Berg *et al.* 1982; Fried & Crothers 1984). According to the 'sliding' model, proteins bind non-specifically to the DNA and subsequently 'slide' along the DNA in search of the specific target site. In contrast, the 'direct transfer' mechanism involves the transient formation of a complex in which the protein is bound to two DNA segments. Upon dissociation of the complex, the protein remains bound to one or the other DNA segment and so migrates in jumps along the DNA. Both mechanisms lead to a reduction of the search volume within the nucleus by reducing a three-dimensional target search to one dimension. We would like to propose an additional mechanism for accelerating the search for specific binding sites: a structural 'indexing' or compartmentalization of the nucleus.

The 'indexes' of the nucleus would be physical clusters of important sequences (binding sites, promoters, enhancers, etc.), which need to be scanned and acted on by various protein factors. Such an index or compartment, rich in specific DNA binding sites, would facilitate the formation of the appropriate DNA–protein complexes required for the control of transcription, replication and for chromosome templating, by reducing a factor's search volume within the nucleus. The close proximity of many regulatory sequences would have a circean effect, effectively caging DNA binding proteins within this compartment. Regulatory proteins could nevertheless traverse the compartment by the direct transfer mechanism, physically scanning the DNA exposed at the bases of loops. Within these compartments one might expect to find both high and weaker-affinity binding sites for factors, which could be important for chromatin 'templating' (Weintraub 1985); that is, the mechanism that copies the protein-structural, epigenetic features of chromatin. If the assembly of active chromatin structure results from the competitive binding of either factors or histones to newly replicated DNA, then the existence of factor-rich compartments could provide a kinetic advantage for the binding of factors over histones during replication. Thus high fidelity transmission of the epigenetic chromatin structure to the daughter cell would be largely a consequence of the compartmentalization of the nucleus.

The channels defined by the bases of loops of one chromosome could be linked to the periphery of the nucleus via the nuclear pores, which may direct and kinetically regulate influx of proteins to the channels. A highly organized nucleus with a fixed orientation towards the cytoplasm, as observed in the *Drosophila* embryo (Foe & Alberts 1985) might permit the relay of spatial information from a structured cytoplasm into the nucleus, to affect gene expression. Finally, if one assumes that the flux of proteins into the nuclear channels is kinetically dependent on nuclear pores, then dissociation of the nuclear membrane lamina–pore complex during mitosis could lead to an automatic loss of these proteins from chromatin. In this light one would not need to propose an additional mechanism for chasing nuclear proteins into the cytoplasm during metaphase.

Our results suggest that the higher-order organization of the nucleus is determined by bases of DNA loops and the proteins that bind to them. If these sites change with the differentiation of a cell and specialization of its pattern of expression, then one would expect that nuclei from different cell types would have different networks and channels. Such a cell-specific three-dimensional organization of nuclei was also discussed in the 'gating' hypothesis of Blobel (1985). Although these predictions have not yet been shown to be correct, with the means to dissect the higher-order chromatin conformation at hand, we may soon get more than a glimpse at chromosomal order.

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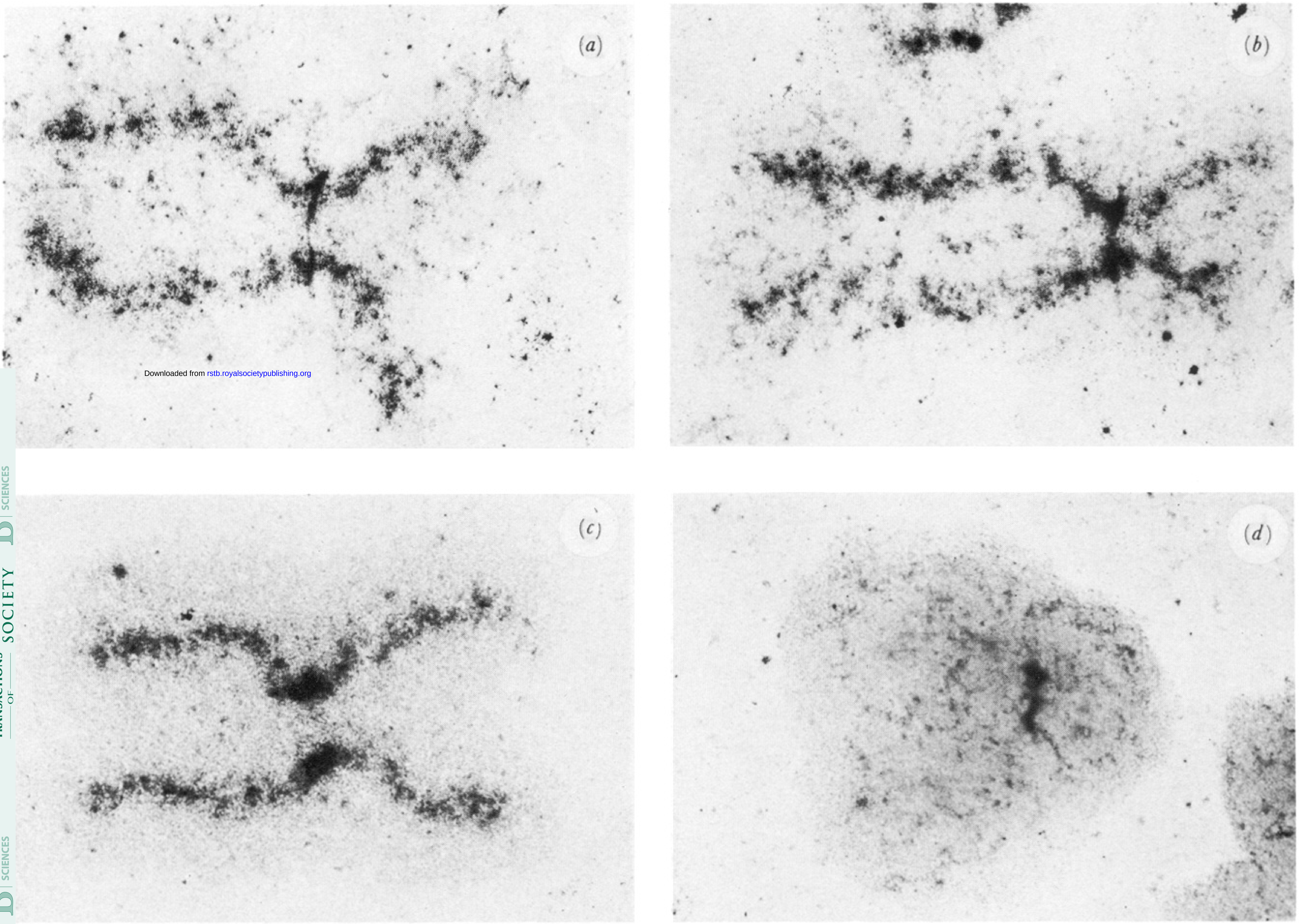

FIGURE 1. Immunolocalization of topoisomerase II in metaphase chromosomes. In (*a*–*c*) the antibody directed against topoisomerase II (Sc1 protein, molecular mass 170000 Da) identifies an axial element that extends the length of the chromatid going through the kinetochore elements. Some of these micrographs provide evidence of the substructural organization of the scaffold, which appears to consist of an assembly of foci, forming in places a zig-zag or coiled arrangement. A peroxidase-coupled secondary antibody was used for the staining reaction (Gasser *et al.* 1986) with both immune (*a*–*c*) and preimmune (*d*) sera. The kinetochore elements, but not the axial staining, are seen in the control (*d*) (Gasser *et al.* (1986), see also Earnshaw & Heck (1985)).