Facilitation of chromatin dynamics by SARs
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Metaphase chromosome condensation is a dynamic process that must utilize cis elements to form and maintain the final structure. Likewise, cis elements must regulate the accessibility of chromatin domains to protein machines involved in processes such as transcription. Scaffold associated regions appear to play important roles in both of these dynamic processes.

 Addresses
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Abbreviations
PEV position effect variegation
SARs scaffold associated regions

Introduction
Various structural studies have demonstrated that the chromosomal architecture of the nucleus is highly ordered and suggest that it might be held in place by some sort of nuclear scaffold. This organization must permit dynamic movements of chromatin during activities such as enhancer looping, recombination and chromosome condensation. The mechanisms whereby nuclear order and chromatin dynamics are achieved remain enigmatic. These biological processes are presumably governed by cis elements that are distributed at intervals along the genome. Activities mediated by such cis elements presumably include tethering chromatin to some kind of nuclear scaffold, serving as signalling or initiator stations from which open, closed or heterochromatic chromatin states can be traced in suitable electron micrographs of histone-depleted metaphase chromosomes [3]. They are possibly best described as being composed of numerous clustered, irregularly spaced runs of As and Ts (A tracts), the non-B DNA structure (narrow minor groove, bends, propensity to unwind) of which are recognized by certain SAR-binding proteins such as histone H1 and topoisomerase II (reviewed in [4]). It has been difficult to address and decipher the in vivo role of SARs because of their non-standard sequence determinants and the absence of a convenient assay system that allows functional studies. Despite these difficulties, the recent observations reviewed here show that progress is being made in understanding SAR function. Structural studies demonstrate that SARs are not arranged randomly but appear juxtaposed in metaphase chromosomes as is expected from the loop-scaffolding model, whereas other studies strongly implicate SARs as genuine cis elements of chromosome dynamics and gene expression.

SARs and metaphase chromosome structure
Numerous structural studies support the view that the chromatin fiber of metaphase chromosomes is organized into loops (reviewed in [5]). If SARs define the bases of the loops in mitotic chromatin and are juxtaposed by the scaffolding, then native chromosomes should have an AT-rich subregion where the SARs ‘queue up’. Using special staining techniques combined with optical sectioning by confocal microscopy and image reconstruction [6], this AT-queue was visualized in the giant chromosome of the Indian muntjac (Figure 1a,b). The AT-queue proceeds through the chromosomal cylinder on an irregular, helical-like path that is stretched longitudinally at certain intervals (Figure 1c).

Remarkably, these structural studies suggest that classic chromosome banding patterns (reviewed in [7]) are manifested by the differential folding pattern of the AT-queue rather than by a large difference in the base composition between Q and R bands (Figure 1c). The Q-bands (also known as G-bands) obtained with classic staining methods appear morphologically ‘AT-rich’ as they are in regions where the AT-queue is more tightly coiled/folded. In contrast, R bands appear morphologically ‘AT-poor’ as they are in regions where the AT-queue is more unfolded and centrally positioned (Figure 1c). R-bands are known to replicate early, to contain most housekeeping genes and are enriched in hyperacetylated histone H4 [8] and DNase I-sensitive chromatin [9]. This suggests they have a more open chromatin conformation, consistent with a central AT-queue with longer loops that reach the nuclear periphery. In contrast, Q-bands contain fewer genes and are proposed to have loops that are shorter and more tightly folded, resulting in an AT-queue path resembling a coiled spring.

Whereas a more extended chromatin conformation is thought to contribute to the longer loops of R-bands, a
greater length of DNA between SARs may also contribute. In support of this view, the 90 kb β-globin locus is in a G-band and has 8 SARs mapped within it, whereas the 140 kb α-globin complex is in an R-band and no SARs are found in this region [10]. Similarly, a study in which bulk SAR and bulk non-SAR DNAs were used to paint chromosomes by in situ hybridization found that G-bands have a higher ratio of SAR to non-SAR DNA than do R-bands [11•].

Topoisomerase II is a major protein of metaphase scaffolds [12,13] that preferentially binds SAR DNA in vitro [14]. The structural studies using intact chromosomes discussed here extend the notion that topoisomerase II follows the scaffold as it was found that it and the AT-queue co-localize [6]. These observations support the view that the scaffolding mediates chromatin-loop formation, presumably through interactions involving or facilitated by SARs.

**SARs and chromosome dynamics**

Are SARs involved directly in chromosome dynamics? The role of SARs in chromosome condensation was tested by using an artificial protein [15] that consists of numerous, linked DNA-binding domains, called AT-hooks, which preferentially interact with A tracts through minor groove
contacts [16]. MATH-20 is a multi AT-hook SAR-binding protein composed of 20 reiterated AT’ hooks. As the numerous A tracts of SARs can simultaneously accommodate all 20 hooks, this protein was found to bind SAR DNA and SAR chromatin with both extremely high specificity and affinity (see Figure 2a).

Assembly of metaphase chromosomes from added sperm or somatic nuclei by *Xenopus* egg extracts is known to go through a number of morphological intermediates to form individual chromatids. A low dose of MATH-20, about one molecule bound per 15 kb of DNA, was found to inhibit a late stage of chromosome condensation. Under these con-

![Figure 2](image)

SARs are necessary for mitotic chromosome assembly [15]. (a) Construction of a highly specific SAR-binding protein. MATH-20 is a multi-AT-hook protein comprising of 20 reiterated AT hook peptide motifs, derived from HMG-I/Y, linked with a spacer of ~26 amino acids. As depicted, this protein is extremely specific for SAR DNA and SAR chromatin, binding with high affinity because other sequences cannot simultaneously accommodate all 20 hooks. (b) MATH-20 blocks chromosome assembly. *Xenopus* egg extracts convert somatic nuclei (left) into metaphase chromatids (top center). Titration of SARs with MATH-20 specifically inhibits this without inhibiting condensation, resulting in abortive mitotic structures where the condensed chromatin is located at the nuclear periphery surrounding a near-empty center thought to arise from the disassembly of nucleoli (bottom right). When added to formed chromatids, MATH-20 interferes with their structural stability and they collapse into individual spheres (‘chromatid balls’; top right).
ditions, chromatids do not form while morphologically aberrant structures that have a mitotic-like extent of compaction accumulate. Using somatic nuclei, aberrant mitotic nuclei accumulate which contain highly condensed chromatin pushed against the periphery surrounding an ‘empty’ center (Figure 2b). MATH-20 does not inhibit early mitotic events such as the breakdown of the nuclear membrane/lamina complex and the disassembly of the nucleolus. When sperm nuclei are used, biochemical remodeling and subsequent incorporation of proteins known to be involved in condensation — such as topoisomerase II [17] and XCAP C/E [18] — are not inhibited by MATH-20. Interestingly, the structural stability of chromatids in mitotic extracts is also affected (i.e. addition of MATH-20 results in an ATP-dependent collapse of formed chromatids into individual spheres [Figure 2b]). It appears that SAR-bound proteins necessary for the structural stability of chromosomes are displaced to non-SAR sequences by MATH-20, resulting in a misdirected condensation reaction leading to the formation of chromatid ‘balls’. Although these observations suggest strongly that SARs are cis elements of chromosome dynamics, the mechanism whereby they exert their role remains speculative. SARs could be the target of binding proteins that either mediate or facilitate the formation and juxtapositioning of metaphase chromatin loops. Important recent progress has led to the identification of a protein complex in Xenopus egg extracts, called condensin, implicated in chromosome condensation [19••]. One subunit of this complex is homologous to the SARs affect gene regulation through chromatin-state transitions. (a) Developmentally regulated opening of the immunoglobulin μ gene utilizes a collaboration between an enhancer and associated SAR. The μ gene has an intronic enhancer with an associated SAR. The enhancer alone forms a DNase I hypersensitive site, but association with a SAR is needed for developmentally regulated general nuclear sensitivity and gene activation during B cell differentiation [27]. In an elegant set of experiments [28••], it was further shown that both accessibility of T7 RNA polymerase to a promoter located 1 kb from the enhancer and regional demethylation require the enhancer and associated SAR (top), whereas the enhancer alone only conferred local accessibility to the adjacent T3 RNA polymerase promoter (bottom). The immunoglobulin κ-chain gene is similarly regulated and also has an intronic enhancer with an associated SAR. A similar study found that both the intronic SAR and associated enhancer are necessary for the developmentally regulated demethylation that precedes transcriptional activation of the κ-chain gene [29,30]. The common cohabitation of SARs and enhancers suggests this could be a general mechanism for conferring specificity to SAR-mediated chromatin state transitions. (b) MATH-20 protein suppresses PEV caused by juxtaposition next to SAR-like Satellite III sequences in Drosophila [32••]. The red pigmentation (black here) of the eyes of wild-type flies requires the protein encoded by the white gene (W+). A chromosomal inversion (white mottled 4 [Wm4]) that places white next to the heterochromatin of the X chromosome results in inactivation of this gene during development in most cells of the eye, although a few small clonal cell populations still express white (center). The presence of MATH-20 in eye cells at the correct point in development restores white expression in the majority of eye cells (at right). Satellite III is a major component of this heterochromatin the properties of which as a SAR have been well studied [45], and this 11 megabase satellite is a target of MATH-20. This targeting presumably interferes with heterochromatinization, preventing the spread of this repressive chromatin state and allowing the white gene to acquire and maintain an active expression state during development.
Drosophila Barren protein, which interacts with topoisomerase II and is required for sister chromatid separation in mitosis [20]. Topoisomerase II preferentially binds SARs in vitro, and immunofluorescence and injection studies suggest that this also occurs in vivo [6,21••]. Topoisomerase II could thus link condensin, via Barren, to SARs whereas MATH-20 could interfere with this targeting. The aberrant mitotic structures induced by MATH-20 addition may be a consequence of such a mistargeting of the condensation proteins to non-SAR sequences.

**SARs and gene expression**

Numerous publications have demonstrated that SARs, in a flanking position, can strongly stimulate the expression of many but not all heterologous reporter genes (reviewed in [4]). Remarkably, this activity is evolutionarily conserved. For instance, yeast SARs flanking a reporter gene increased expression levels 24-fold in tobacco plant cell lines [22]. The SAR effect is only observed following stable integration into the genome in all biological systems tested [22–24]. These cis-acting elements hence appear to require a chromatin environment as transiently transfected DNA is known to be poorly organized into nucleosomes. The evolutionary conservation of the SAR effect on gene expression suggests that conserved structural components of chromatin, rather than species-specific factors, are involved.

An extended working model [4,25] aimed at explaining the general stimulatory role of SARs suggests that their clustered A tracts competitively bind two types of proteins. One type of protein (exemplified in vitro by histone H1 or topoisomerase II) bind SARs or certain AT-rich satellites cooperatively and lead to compaction of the chromatin fiber, chromosome condensation (looping) or heterochromatin formation. Conversely, other proteins such as HMG-I/Y and the larger derivative MATH-20 bind non-cooperatively to SARs and can displace the compacting proteins by disrupting their cooperative interactions, hence resulting in chromatin accessibility (or misguided compaction during metaphase). This simple model is based on in vitro competition experiments demonstrating that HMG-I/Y and the peptide antibiotic distamycin, which selectively bind the minor groove of A tracts, specifically make SAR-containing DNAs accessible by displacing pre-bound histone H1 from SARs [26]. In vivo support originates from the observation that distamycin added to cells increases the accessibility of the internucleosomal linker DNA of SARs but not of hypersensitive sites [25].

This model is consistent with studies demonstrating that the developmentally regulated access of the immunoglobulin μ gene utilizes a collaboration between an enhancer and an associated SAR [27,28••]. In an elegant set of experiments, it was shown that both the SAR and the enhancer were required to spread the accessibility to a T7 RNA polymerase promoter located 1 kb away, whereas the enhancer alone could only confer local accessibility to an adjacent T3 RNA polymerase promoter (Figure 3a). A related study [29] found that both the intronic SAR and associated enhancer of the κ-chain gene are necessary for the extended, developmentally regulated demethylation that precedes transcriptional activation of this gene. In this case, it was shown that SARs from Drosophila or plants could functionally substitute for the endogenous SAR, indicating that a general property of SAR DNA rather than a specific sequence is important [30]. The frequently observed association of SARs and enhancers [31] might confer specificity to the initiation of chromatin opening.

Another example of SAR-mediated gene activation is the suppression of position-effect variegation (PEV) of the white mottled allele by the artificial, SAR-specific MATH-20 protein in transgenic Drosophila (Figure 3b; [32••]). This PEV is caused by a chromosomal inversion that places the white gene near the centric heterochromatin of the X chromosome. The resulting stochastic inactivation of this gene is supposedly caused by cooperative spreading of heterochromatin into the juxtaposed euchromatic region. SAR-like Satellite III sequences are a major component of this centric heterochromatin [33] and they are a target of MATH-20. The simplest interpretation follows the model discussed — that is to say, MATH-20 bound to the 11 megabases of Satellite III sequences is proposed to interfere with the formation and cooperative spreading of heterochromatin in this region, thus reducing the number of cells in which the rearranged white gene has an inactive chromatin structure.

An alternate model proposes that SAR function is mediated by sequences that readily unwind under torsional stress [34]. To test this proposal, an oligonucleotide containing an SAR-derived base-unpairing region was mutated to stabilize base pairing. In contrast to the wild-type oligonucleotide, the concatenated mutant had reduced association with nuclear matrices and did not stimulate gene expression of a chromosomally integrated reporter gene. These mutations, however, also disrupted A tracts so this, rather than or in addition to the loss of base-unpairing potential, could account for the observed effects. Several proteins have been described that bind to base-unpairing regions of SARs, apparently as double-stranded DNA [35•,36–40]. At least some of these are implicated in gene regulation, are restricted to certain cell types and at least partially partition into nuclear matrices. Perhaps these proteins target certain SARs to confer specificity to gene activation similar to the cases described above.

**Conclusions**

In summary, SARs are cis elements of chromosome dynamics. They play a global role during mitotic chromosome condensation and are also necessary for the maintenance of chromosome structure. The reader should note that it remains to be demonstrated that they are direct binding sites for protein complexes involved in condensation, such as condensin.
SARs are also implicated in gene expression, by facilitating both the transition and the spreading of an open chromatin structure. Another chromatin-specific element might interrupt the spread of chromatin accessibility or inaccessibility initiated by SARs, thus determining domain boundaries. Such boundary elements have been shown to restrict enhancer interactions to intra-domain gene activation [41–43]. Although the model of SAR function discussed is well supported by different lines of experiments, however, other explanations cannot be excluded. For example, SARs could be preferentially targeted by the nucleosome remodeling factor CHIRAC via topoisomerase II, which is a subunit of this complex [44].

It is probable that different complexes will be found to target SARs to achieve dynamic transitions in chromatin structure. Much work remains to be done to elucidate the relationship between SAR function and nuclear organization.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:• of special interest** of outstanding interest


11. Craig JM, Boyle S, Perry P, Bickmore WA: Scaffold attachments• within the human genome. *J Cell Sci* 1997, 110:2673-2682. Chromosomes were painted by *in situ* hybridization using total 'attached' and 'loop' DNA fractions prepared from nuclei or metaphase chromosomes by the scaffold, matrix or nuclear skeleton protocols. Both types of scaffold DNA preparations indicated that gene-poor Q-bands are enriched ~2-fold for SARs relative to gene-rich R-bands. The ratios obtained for matrix and skeleton preparations varied depending on whether the DNA fractions were prepared from nuclei or chromosomes, indicating that these attachments are more dynamic.


XCAP-C/E (Sccl) and topoisomerase II (Scll) were the most abundant proteins present in chromosome scaffolds as originally prepared by Laemmli and collaborators [13]. As was previously shown for topoisomerase II, this important paper demonstrates that XCAP-C/E, in a complex that includes a homolog of the *Drosophila* Barren protein, is required for chromosome condensation in *Xenopus* egg extracts. The targeting of condensin complexes to chromosomes is regulated during the cell cycle by phosphorylation. Condensin and topoisomerase II can be targeted to chromosomes independently of each other, but these results do not rule out a targeting of condensin to SARs by interactions with topoisomerase II. Such interactions could be mediated by the Barren protein (see [20]) and could be affected by phosphorylation and, hence, be transient.


This study used high-resolution confocal microscopy to observe the three dimensional motion of interphase chromatin in living cells. Consistent with the concept of a highly defined nuclear architecture, large-scale movement was not observed but segments of chromatin are free to move within their local region which represents ~1% of the nuclear volume. This constrained diffusion appears to be caused by Brownian motion rather than by an active process. The motion observed in *Drosophila* nuclei utilized chromatin labelled with microinjected, fluorescently labelled topoisomerase II which was found to concentrate on Satellite III sequences which are essentially an 11 megabase SAR. It is probably that topoisomerase II bound other SARs too but their sizes were too small to give a detectable fluorescent signal.


23. Klehr D, Maass K, Bode J: Scaffold-attached regions from the human interferon β domain can be used to enhance the stable expression of genes under the control of various promoters. *Biochemistry* 1991, 30:1284-1270.


27. Forrester WC, van Genderen C, Jenuwein T, Grosschedl R: Dependence of enhancer-mediated transcription of the


The members of this laboratory demonstrated previously that an intronic enhancer plus its associated SAR function together to allow developmentally regulated gene activation. Here they extend this work by utilizing bacteriophage T3 and T7 RNA polymerases to probe the accessibility of chromatin in isolated nuclei. They show that an intronic enhancer can only open chromatin locally to allow access to an adjacent T3 promoter. In contrast, the enhancer plus its normally associated SAR opens a larger region of chromatin to allow access to a 1 kb distant T7 promoter.


Using an artificial SAR-specific DNA binding protein (MATH-20) composed of reiterated AT-hooks derived from HMG I/Y, inactivation of a gene placed next to heterochromatin is suppressed. A major component of this heterochromatin is an SAR-like satellite of 11 megabases that is a target for MATH-20. Binding by MATH-20 apparently interferes with heterochromatinization of satellite III sequences, and presumably prevents spreading of this inactive chromatin state to encompass the rearranged gene.


The authors of this paper claim that a cell-type specific protein, SATB1, targets a SAR to the nuclear matrix – resulting in gene inactivation.


