Chromosome dynamics: **Fuzzy sequences, specific attachments?**
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The assembly of condensed chromosomes in a cell-free system is inhibited by the addition of proteins that bind AT-rich DNA. Does this implicate the AT-rich scaffold attachment regions (SARs) in the formation of chromosomes?

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The assembly of mitotic chromosomes from interphase chromatin is one of the least understood phenomena of cell division. Simple measurement of the length of individual chromosomes indicates that chromosome condensation results in a roughly 10 000-fold linear compaction of the DNA helix. In addition, comparison of the genetic and spatial relationships of different loci suggests that the linear organization of genetic loci on genomic DNA is maintained within the condensed chromosome. Biochemical and ultra-structural analyses suggest that the chromosome is assembled through a hierarchy of folding steps. The 2 nm DNA double helix winds around complexes of histone proteins to form an 11 nm nucleosome fiber. This then folds into a thicker, 25–30 nm fiber, and this '30 nm' fiber then assembles into a series of 'higher-order' structures. The details of the higher-order structures and the mechanism of their assembly remain unknown and controversial.

Almost twenty years have passed since a now classic series of papers examined the structure of histone-depleted chromosomes [1–3]. Electron micrographs showed a residual proteinaceous core sitting within a cloud of DNA, with an overall shape similar to that of an unextracted chromosome. The protein core lay at the base of DNA loops (Fig. 1). These results suggested that the core might contain factors important for chromosome architecture, in a structure termed the 'chromosome scaffold'. This led to the suggestion that a fundamental aspect of higher-order chromosome structure might be the binding of specific DNA sequences to a subset of chromosomal proteins that form the chromosome core: upon extraction of the histones, regions in between sequences bound to the chromosome core would extend away from the core and generate the observed DNA loops.

Subsequent experiments identified a number of DNA sequences that fractionate with the scaffold *in vitro* (reviewed in [4]). These scaffold-attachment regions (SARs; also called matrix-attachment regions, MARs) were generally 0.5–1 kilobase sequences rich in the bases A and T, with long dA.dT homopolymer stretches, and were often located in the regulatory regions flanking protein coding sequences. The identification of numerous SARs, however, failed to indicate a strict consensus sequence. SARs are therefore called 'fuzzy' DNA elements: the specificity of the scaffold–SAR interaction is thought to be mediated by DNA-binding proteins that recognize structural features of the DNA — within its narrow minor grooves — rather than specific DNA sequences [4]. Recently, the sites of AT-rich sequences in mitotic chromosomes were found to be consistent with the scaffold–SAR hypothesis illustrated in Figure 1 [5], but it is not yet known whether the large amount of AT-rich DNA found throughout the genomes of higher eukaryotes binds to the chromosome scaffold and has SAR function.

Characterization of the protein components of the chromosome scaffold originally identified two proteins, ScI

Figure 1
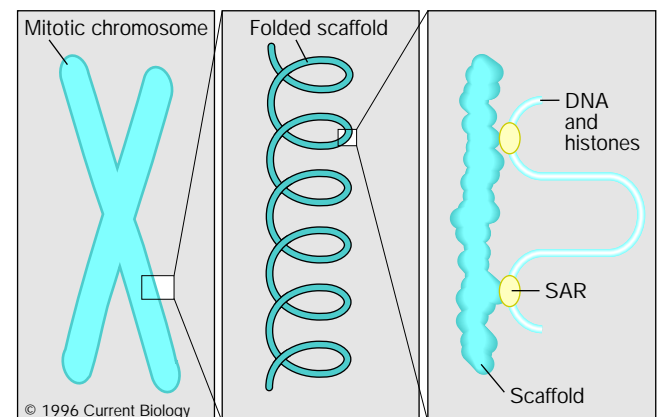
A section of a mitotic chromosome is expanded to show the mechanism of folding proposed by the scaffold–SAR model of chromosome structure. Scaffold attachment region (SAR) sequences bind to chromosome scaffold proteins. Non-SAR DNA loops out from the scaffold upon histone extraction. In the intact chromosome, non-SAR DNA is presumably assembled into higher-order structures. The scaffold itself folds, possibly by helical coiling, to give rise to the mitotic chromosome. If SARs function as global structural elements of the chromosome, they should be found at the base of most DNA loops. The distribution of AT-rich DNA in mitotic chromosomes has recently been examined by confocal microscopy using daunomycin, a drug that exhibits a large increase in fluorescence upon binding very AT-rich DNA [5]. The distribution of AT-rich sequences was found to be consistent with the suggestion that AT-rich sequences are enriched at the base of DNA loops. For a full description of the SAR–scaffold model, see [4].

and ScII, as major components [6]. ScI was found to be DNA topoisomerase II, a DNA strand-passing enzyme [7,8]. ScII was recently cloned and identified as a member of the SMC (structural maintenance of chromosomes) family of structural chromosome proteins [9,10]. Subsequent studies have identified a number of other proteins that bind to SARs, at least *in vitro* — notably histone H1 and the 'high mobility group' nuclear proteins HMG-I/Y [11,12]. Although many SARs and SAR-binding proteins are known, confirmation of the scaffold–SAR hypothesis will require answers to two questions. First, is a specific DNA element involved in global higher-order chromosome architecture, and if so, is this element a SAR? And second, what proteins bind to SARs specifically and serve as DNA-loop fasteners *in vivo*?

In a recent paper, Strick and Laemmli [13] have taken a step toward answering these questions. One prediction of the scaffold–SAR hypothesis is that disruption of the interaction between SAR-binding proteins and SARs should cause global disruption of chromosome structure. Strick and Laemmli tested this prediction by examining the effects of synthetic SAR-binding proteins on chromosome assembly. Synthetic proteins were generated by making oligomers of the AT-binding domain of the human HMG-I protein. This domain binds selectively to the minor groove of dA.dT homopolymers and so has been called the 'AT hook' [14]. HMG-I has three AT hooks that appear to bind cooperatively to AT-rich DNA with a dissociation constant (K_d) of 1 nM. The specific function of HMG-I in chromosome organization is not known. A synthetic multiple AT-hook (MATH) protein with 20 AT hooks ('MATH20') was shown by Strick and Laemmli to have AT-sequence specificity with an impressive K_d of 2.6 pM. MATH10 (with 10 AT hooks) showed similar sequence specificity but an intermediate K_d of 18.2 pM.

Can MATH proteins bind specifically to SARs in the context of chromatin? To address this question, Strick and Laemmli [13] immobilized MATH11 on beads and added the beads to partially digested *Drosophila* chromatin. When the DNA bound to the MATH11 beads was analyzed, the SAR from the *Drosophila* histone locus was found to be enriched in the MATH11-bound fraction, but non-SAR DNA from within this locus was not enriched. It is known that nucleosomes cover this SAR *in vivo* [15], but given such strong binding affinity, it seems possible that MATH proteins displace histones from SARs. These results demonstrate the strong sequence preference of MATH proteins for AT-rich DNA containing dA.dT homopolymers. But it is possible that MATH proteins can bind non-SAR AT-rich DNA as well as biochemically defined SARs.

What role do AT-rich sequences have in chromosome dynamics? Strick and Laemmli [13] used cytoplasmic extracts from *Xenopus* eggs to test the effects of MATH proteins on chromosome assembly and structure. These extracts are prepared so that they contain high levels of the cdc2 kinase activity that drives cells into mitosis; they therefore convert added nuclei into mitotic chromosomes [16,17]. When MATH20 was added along with sperm nuclei into such a chromosome assembly extract, the formation of chromosomes from the nuclei was inhibited in a dose-dependent fashion (Fig. 2). Other potential inhibitors, such as histone H1 and polylysine, had no effect. MATH10 also inhibited chromosome assembly from sperm nuclei, but at an increased dose that was approximately proportional to its lower dissociation constant. These results suggest that MATH proteins affect chromosome assembly in a stoichiometric fashion.

To eliminate the possibility that the effects of the MATH proteins were specific to the assembly of chromosomes from sperm chromatin, somatic nuclei from cultured L cell fibroblasts were used in the same assay (Fig. 2). In the presence of MATH20, chromatin appeared to condense on the periphery of L cell nuclei and produced an unusual chromatin ring structure, but individual chromosomes were not formed. MATH20 added to chromosomes formed from L cell nuclei caused the collapse of these chromosomes into spherical balls of chromatin. The formation of ring-shaped nuclei and disruption of preformed chromosomes was blocked by the addition of excess SAR DNA, but not by non-SAR DNA. Together, these results suggest that the activity blocked by MATH proteins is required for the formation and maintenance of chromosome structure. The suppression of MATH activity by addition of exogenous SAR DNA supports the conclusion that the MATH proteins disrupt chromosome structure by interfering directly with protein–DNA interactions required for global chromosome structure. Interestingly, MATH20 added to an interphase cytoplasmic extract had no effect on interphase L cell nuclei, consistent with the suggestion that the organization of higher-order chromatin is distinct between interphase and mitosis.

Which of the protein–DNA interactions required for mitotic chromosome organization are affected by added MATH proteins? If exogenously added MATH20 occupies SARs in the context of the chromosome, as predicted, then it should behave as a competitive inhibitor of the scaffold proteins that normally bind SARs. Strick and Laemmli [13] showed, however, that the major protein components recovered with chromosomes do not change even when the amount of added MATH20 far exceeds that required for full inhibition of chromosome assembly. These major components include topoisomerase II and the *Xenopus* SMC family homologues XCAP-C and XCAP-E, all of which are known to be required for chromosome condensation in this *in vitro* system and fractionate with the chromosome scaffold in other systems

Figure 2
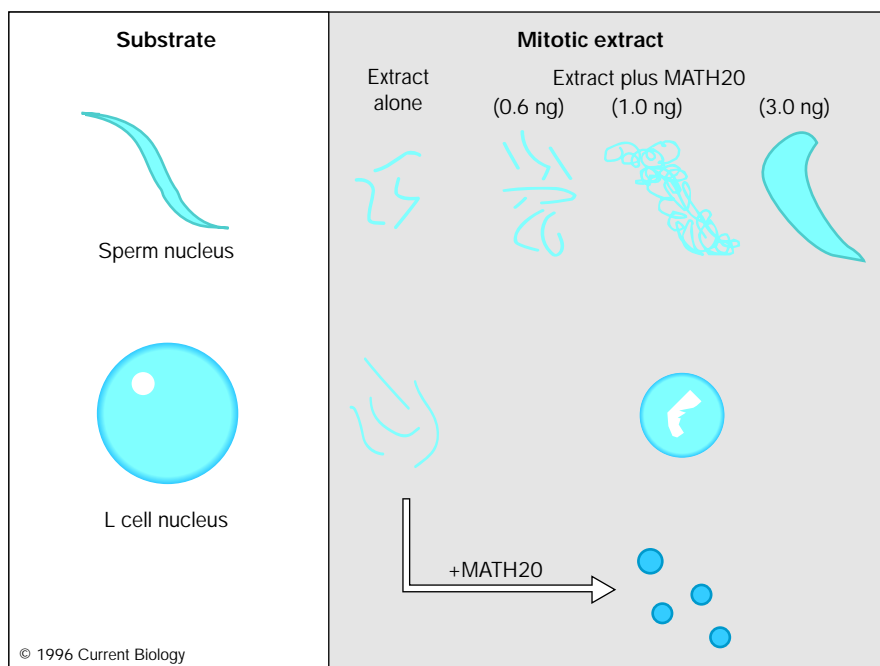
The effects of MATH proteins on chromosome assembly *in vitro*. The morphologies of products assembled *in vitro* in the absence or presence of MATH proteins are shown [13]. Sperm nuclei form mitotic chromosomes in a mitotic *Xenopus* egg extract. In the presence of small amounts of MATH protein, individual chromosomes are formed. Increasing amounts of MATH protein progressively inhibit assembly of defined fibers until condensation is completely inhibited. MATH proteins also inhibit the formation of chromosomes from L cell nuclei in the same extract. The resulting nuclei are smaller than those used as the input substrate, and the chromatin appears condensed on the nuclear periphery. Holes in the condensed chromatin are likely to be the remnants of nucleoli that have been disassembled in the mitotic extract. If MATHs are added to the reaction after the formation of chromosomes from L cell nuclei (arrow), the chromosomes collapse into dense balls of chromatin.

[9,16,18,19]. Strick and Laemmli [13] propose, therefore, that the inhibition of chromosome assembly and disruption of preformed chromosomes reflect the displacement of scaffold proteins to non-SAR locations, causing the formation of 'incorrect' associations between non-SAR DNA and the scaffold. The chromatin then condenses, but the formation of individual, axially extended chromosomes is inhibited. This is a reasonable model, but an alternative interpretation is also possible. The multiple AT-binding domains of MATH proteins might themselves generate tightly bound, inappropriate chromatin cross-links that inhibit, yet are distinct from, the chromosome condensation process. This would produce a dose-dependent relationship between the amount of MATH protein added and the degree of inhibition of chromosome condensation. Characterization of the binding sites in chromosomal DNA for MATH proteins, and finding where the MATH proteins go within chromosomes, should distinguish between these two possibilities. It would also be interesting to know whether MATH20 changed the salt extractability of topoisomerase II, XCAP-C and XCAP-E, or any other chromosomal proteins. In addition, we need to know what roles, if any, a *Xenopus* HMG-I homologue or other endogenous AT-binding proteins play in the *in vitro* chromosome assembly system.

Strick and Laemmli [13] propose a specific role for SARs in global chromosome architecture. Confirmation of this model will require further characterization of the binding sites for MATH proteins within the chromosome. Given the large amounts of AT-rich satellite DNA in higher eukaryotes, it seems possible that MATH proteins might bind some of these sequences. How many of these satellites are actually SARs — that is, DNA sequences that localize to the base of DNA loops and bind to or fractionate with chromosome scaffold proteins — is not known. Nonetheless, the effects of MATH proteins in a functional assay for chromosome assembly make a strong argument that AT-rich DNA plays a role in the organization of condensed chromosomes.

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