Packaging genes into chromosomes

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Né en 1940, M. Ulrich Laemmli a fait des études de physique du solide à l'Ecole polytechnique fédérale de Zurich, puis s'est orienté vers la biologie moléculaire, domaine dans lequel il a fait sa thèse de doctorat à l'Université de Genève. Professeur à l'Université de Princeton, il revient à Genève, en 1980, où il est nommé professeur au département de biochimie et au département de biologie moléculaire de la Faculté des sciences de l'Université. Il dirige aujourd'hui ce dernier département.
Introduction

Chromosomes have fascinated biologists for over 100 years, ever since optical microscopes approached their theoretical resolving power and specific stains became available to highlight subcellular structures of dividing cells. Armed with these tools, it became evident to biologists that cells undergo cyclic morphological changes, manifested by the disappearance of the nucleus followed by the emergence of intensely staining bodies. The latter were called chromosomes (chromos=color; soma=body), consisting of a substance called chromatin (literally colored material). Chromosomes are invisible in the interphase nucleus, thin during their initial appearance and then thicken to rod-shaped structures as the cell prepares for division. Early pioneers of this period included cytologists Theodor Boveri, Karl Rabl and the first chromatin biochemist Friedrich Miescher, who succeeded in separating chromatin into deoxyribonucleic acid (DNA) and its associated basic proteins.

It required about a century of fascinating scientific activity to link chromosomes to inheritance and genes, to establish genetic maps, to connect genetic activity via a messenger to protein synthesis, to unravel the genetic code, to demonstrate the double helical structure of DNA and to understand the principles of the semi-conservative nature of replication of the DNA double helix.

The two most important tasks a cell has to accomplish are to duplicate its genes with great precision and to assure the perfect distribution of identical duplicated sets of chromosomes to each daughter cell. Maintenance of the genetic information by high fidelity replication is based on elaborate proofreading processes during DNA replication and a complex repair machinery that detects and corrects DNA lesions introduced post-replication, e.g. by UV light. To assure perfect distribution of the duplicated genes, the cell wraps the long DNA thread ever so carefully prior to cell division into highly compact genetic delivery parcels, the chromosomes. Chromosomes are composed of two copies of the genome called chromatids that are held together at a subregion called the centromere, resulting in an X-shaped appearance. The condensation of chromosomes serves to untangle the extended chromatin fiber of each chromosome of the interphase nucleus and to allow attachment of the spindle for subsequent segregation to the daughter cells.

Chromosome condensation is a formidable topological problem which has fascinated us for a number of years. Our laboratory has focused its attention on the general structure of metaphase chromosomes and nuclei, with the long term goals of elucidating the mechanisms of chromosome dynamics and understanding the relationship between chromosome structure and gene activity.

Compacting DNA into chromosomes: DNA loops of histone-depleted chromosomes

The haploid human genome contains over a meter of DNA distributed into 23 chromosomes. Elegant viscometry studies revealed a single DNA molecule per chromatid [1]. This single, giant molecule is shortened in length about 10,000 times by a hierarchy of folding levels prior to cell division during mitosis. The initial two levels of DNA packaging generate the basic chromatin fiber. First, a fixed amount of 146 bp of DNA is wrapped around the disc-shaped nucleosomes which are the repeating subunits of the chromatin fiber and constructed from the four basic core histones. Second, nucleosomes, strung along the DNA akin to pearls, assemble in turn into the basic chromatin fiber, probably by a helical arrangement. The so-called linker histone H1 structurally stabilizes this fiber by forming interior crossties between the DNA that links nucleosomes. The basic chromatin fiber is generally considered to be transcriptionally inactive in its folded form and much scientific attention is focused toward an elucidation of the mechanism whereby this fiber is rendered competent for genetic activity. The activation
process is accompanied by unfolding of the fiber, chemical modifications and dissociation or unraveling of certain strategically located nucleosomes. This chromatin opening process serves to facilitate the binding of gene regulatory proteins to their DNA target sequences to ensure correct gene expression (reviewed in [2]).

The basic chromatin fiber shortens the DNA in length by a factor of roughly 36, falling far short of the final packaging ratio of 10,000 in chromosomes. Prevailing models in the 70s suggested that further compaction is achieved by a general modification of the chromatin fiber, e.g. phosphorylation of histone H1 to create a mitotic fiber that somehow self-assembles hierarchically into coils and/or folds. We initiated our work on chromosomes with an alternative idea by proposing that chromosome structure is brought about by a special set of non-histone proteins, called scaffolding proteins, that interact specifically at certain DNA regions to fold the fiber into loops. These regions were later called SARs for scaffold associated regions. The scaffold proteins acting at SARs were proposed to bring about the morphological changes of chromosomes by close packing of preexisting and/or de novo formed loops by some kind of higher-order assembly process [3].

Support for this notion was based on an extraction procedure that selectively removed histones from chromosomes but maintained the scaffold interactions; the latter interactions turned out to be more stable under certain experimental conditions. Histones were gently extracted from chromosomes by competition with an excess of the polyanions dextran sulfate and heparin in a low ionic strength buffer. During this process, the positively charged histones exchange from DNA to the highly negatively charged polyanions which are in vast excess over the also negatively charged DNA [fig. 1].

Amazingly chromosomes did not fall apart into linear DNA, despite complete extraction of histones and many but not all non-histone proteins. Instead, they appeared as very expanded chromosomes with the shape and fragility of butterflies [fig. 2]. The histone-depleted chromosomes contained a halo of DNA loops of about 50 to 100 kb in size that were attached to a central, chromosome-shaped substructure called the scaffold. Our early observations suggested that chromosome structure may be brought about by folding of the chromatin fiber into 50 to 100 kb-long loops held together by a longitudinal network of scaffolding proteins. Since scaffolding proteins may have a shape-determining role during condensation and may also serve as a structural skeleton for the final structure, we used the term scaffolding to encompass both possibilities [4].

The loop/scaffold model discussed above is based on observations made with extracted, unfolded chromosomes. Does this model apply to the native structure? Many years of work were necessary to extend the loop model to native chromosomes successfully, and much more research will be needed to understand the molecular mechanisms of chromosome dynamics.

**Chromatin loops**

If the DNA loops observed following histone depletion are reflective of the native structure then one should observe shorter chromatin loops in unextracted chromosomes. The difficulty of observing such loops arises from the compactness of chromosomes that makes individual strands difficult to trace. But we noticed that a minor reduction of the divalent cation concentration led to a reversible separation of the chromatin strands by charge repulsion. This facilitated the examination of the inner order of chromosomes, and the resulting studies by electron microscopy of thin-sectioned or surface-spread, swollen chromosomes provided ample evidence for chromatin loops [fig. 3]. Importantly, these microscopy studies provided independent evidence for some kind of tethering device – scaffolding – which holds the chromatin fiber in loops [5].
Fig. 1 Morphological and biochemical dissection of metaphase chromosomes.

A) Each chromatid contains a single DNA molecule that is shortened in length about 10,000 fold. This high level of compaction is achieved by a hierarchy of folding principles. One of the final levels of compaction occurs by formation of loops imposed by a set of non-histone proteins that make the chromosomal scaffold. Early evidence for a scaffold/loop model came from observations that quantitative removal of all histone and many non-histone proteins did not unfold the chromosomal DNA completely. Instead, histone-depleted structures were obtained with the general shape of very expanded chromosomes [fig. 2]. The scaffold may be best described as a network of proteins that crosstie the loops. The precise structural relationship of neighboring loops is not known. Topoisomerase II and protein SC2 (XCAP C&E) are prominent components of the scaffold; they are required for chromosome assembly (see text). SARs are highly AT-rich DNA regions of 1-2 kb that define the bases of the DNA loops. Using specific superSAR-binding proteins called MATH [fig. 5], we could later demonstrate that these elements participate in chromosome dynamics, that is, chromosome assembly and structural maintenance [fig. 6].

B) This panel depicts a cross-section of a chromatid. If the highly AT-rich SARs are juxtaposed by the scaffold in native chromosomes then one would expect to observe an AT-rich subregion inside chromosomes (yellow) where SARs queue up (called AT-queue). Three-dimensional reconstruction of chromosomes, selectively stained for SAR or non-SAR (bulk) DNA, confirmed this prediction [fig. 4].

C) This panel depicts a hypothetical loop stained selectively with fluorescent dyes. It serves to explain the techniques used to develop the model shown in figure 5: daunomycin, whose fluorescence emission is highly enhanced by AT-rich DNA, was used to highlight SARs. In contrast, the general DNA dye YOYO quenched with methylgreen was used to highlight the loop bodies. These staining techniques were applied to the very large Indian muntjac chromosomes. The loop size of these chromosomes appear to be large enough to optically resolve the loop base from its body.
Fig. 2 Histone-depleted chromosomes retain the shape of a highly expanded chromosome: early evidence for the scaffold/loop model. Early evidence for a chromosomal scaffold was based on observations that complete extraction of the histones and many, but not all, non-histone proteins led to a dramatic unfolding of chromosomes. The resulting histone-depleted structures retained the general shape of very expanded, fragile chromosomes. Morphologically, they consist of large halo of DNA made up of 50-100 kb loops that are held together by an X-shaped network of protein called the scaffold. These early observations suggested that a final level of chromosome organization is brought about by a set of scaffolding proteins that interact at certain DNA regions, later called SARs, to form loops. In turn, the bases of the loops are somehow crosstied together into a scaffold which may play a role in shape determination during chromosome assembly, but may also serve as a structural support for the final structure [4]. The insert shows a scaled unextracted chromosome to underscore the dramatic expansion occurring during extraction of the histones. Scale 2 μm.
Evidence of chromatin loops in unextracted chromosomes. It was of importance to demonstrate loops at the chromatin level in unextracted chromosomes. We developed several techniques for this purpose.

A) Human chromosomes were spread at low ionic strength resulting in the unfolding of the basic chromatin fiber to its more extended form. These unfolded fibers are visible in the micrograph as peripheral loops [28]. Only those loops that remained absorbed by the microscope grid retain the extended form and are visible at the periphery, the others retract back during the staining procedure into the dark, X-shaped chromosomal body.

B) The surface structure of a chromosome observed with the scanning microscope is also consistent with chromatin loops. The surface was found to be studded with knobs that represent the tips of the chromatin loops; this notion was verified by tangential thin-sectioning [5].

C) A cross-section of a human chromosome shows radial loops of the basic (not extended) chromatin fiber. Loops emanate from a central region where they appear tethered by an organizer, the scaffolding. Note that not all loops can be tracted completely, due to contaminating material adhering to the chromosomal periphery. Also, despite a predominantly radial orientation of the loops, certain fibers run out of the plane of cross-sectioning.
Specific scaffold proteins

Are the chromatin loops tethered by specific proteins and do these proteins interact at specific DNA regions? Indeed, although necessitating a number of years of research, we were able to successfully answer both questions. The difficulty of finding specific scaffold proteins was formidable, but we made an important observation that allowed us to identify potential scaffolding proteins biochemically with some confidence. We noted that certain isolation procedures yielded “soft” chromosomes containing unstable scaffolds where histone-depleted chromosomes fall apart, while another procedure produced “robust” chromosomes with a stable scaffold. Based on the assumption that scaffold proteins were solubilized from soft but not from robust chromosomes during the extraction procedure, we could identify two candidate scaffold proteins by comparative biochemistry of the two different chromosome preparations. Importantly, these proteins, called SCI and SC2 were sufficiently abundant (about two to three per loop) to possibly play a scaffolding role [6].

Both assignments turned out to be correct. SCI was identified in two laboratories as topoisomerase II and was found by immunofluorescence to localize to the chromosomal scaffold [7,8]. Moreover, we established directly that topoisomerase II is necessary for chromosome assembly. It is possible to assemble mitotic chromosomes in vitro in extracts prepared from eggs of the African frog Xenopus laevis. Using such extracts immuno-depleted for topoisomerase II we observed that chromosomes did not condense, but condensation could be restored by addition of purified topoisomerase II [9]. SC2 belongs to a family of proteins which includes proteins XCAP C&E. These proteins are known to be involved in chromosome condensation and also to immunolocalize to the chromosomal scaffold [10,11]. Interestingly, these proteins appear to have a protein motor motif suggesting that condensation may be brought about by “walking” proteins.

Specific loop attachment regions: SARs

SARs, for scaffold associated regions, are DNA segments that define the bases of the DNA loops in vitro. Such specific attachment fragments were required by the loop model and, given their potential importance, we tinkered for many years in search of a methodology to identify attachment fragments. We finally succeeded by using an “exotic” compound, lithium di-iodo salicylate (LIS), to extract chromosomes and nuclei. At very low ionic strength LIS removes all histones and many non-histone proteins but maintains (or allows reformation of) the specific interaction of SARs with the residual nuclear/chromosomal scaffold [12,13]. Many laboratories adopted this methodology and numerous SARs in different biological systems, from plants to human, have been mapped (reviewed in [14]).

SARs are about one kb in size, highly AT-rich and are best described as being composed of numerous A-tracts (AT-rich sequences containing short polymeric runs). Interestingly, their specific interaction with nuclear scaffold proteins is not determined by a precise base sequence but by the altered DNA structural features of the A-tracts, such as their narrower minor groove and DNA bends. The importance of the A-tracts in determining the specificity of the SAR-scaffold interaction is based on experiments using the drug distamycin. This peptide antibiotic binds the narrower minor groove of A-tracts and was found to inhibit all known preferential biochemical interactions of proteins with SARs [15-17]. We call SARs fuzzy DNA elements since they are defined by the numerous, irregularly-spaced A-tracts rather than by a specific sequence.

SARs appear to have a dual structural and functional role in gene expression. They are frequently observed in close association with enhancer elements [13,19]. Many laboratories have observed that SARs, in flanking positions, stimulate expression of various heterologous reporter genes in different biological systems when integrated into the genome,
but not in transient assays [18,29] (reviewed in [14]). We have studied the mechanism of this stimulatory role of SARs and collected solid evidence for a model that describes SARs as regions of chromatin that more easily unfold due to a facilitated displacement of histone H1 as mediated by factors such as HMG-I/Y. The unfolded, more open chromatin may in turn promote the entry of factors necessary for DNA processes such as transcription, methylation or, at mitosis, chromosome condensation [20].

**Our current chromosome model**

If SARs define the bases of chromatin loops and if they are juxtaposed by the scaffolding, then one would expect an AT-rich subregion in native chromosomes where SARs queue up, called AT-queue [fig. 1]. To reveal the AT-queue, we developed special techniques that used the highly AT-specific dye daunomycin to stain SARs at the bases of the loops, and conversely, the general DNA dye YOYO quenched with methylgreen to highlight the loop bodies; methylgreen binds to AT-rich DNA and quenches emission of YOYO from such regions.

Optical sectioning of selectively stained Indian muntjac chromosomes by confocal microscopy and image reconstruction led to the following model of native chromosomes [21] in which the AT-queue is depicted in yellow to contrast it from the green loop bodies [fig. 4]. The AT-queue proceeds through the chromosomal cylinder on an irregular, helical-like path, a bit akin to a coiled spring that is stretched longitudinally at certain intervals. Although individual loops cannot be observed by confocal microscopy, they are graphically indicated in an oversimplified fashion in the model based on a number of considerations. We do not actually know the precise packaging mode of the loops along the AT-queue.

Most satisfactorily, our results also explain an old mystery: the classic phenomenon of chromosome bands. They arise from the irregular folding path of the AT-queue and are a consequence of the loop model [fig 4]. Our model claims that Q-bands (also known as G-bands) correspond to regions where the AT-queue is more tightly coiled/folded. In contrast, R-bands correspond to regions where the AT-queue is more unfolded and centrally positioned. R-bands are known to replicate early and to be genetically more active. Specifically, R-bands contain most housekeeping genes and are enriched in hyper-acetylated histone H4 [22] as well as DNAase I-sensitive chromatin [23] which is reflective of a more open chromatin conformation. In accordance, our model requires that the loops of R-bands are longer in order to reach the chromosomal periphery, either at the DNA level or by having a more extended, open chromatin configuration, or both. In contrast, the loops of the Q-bands contain fewer genes and are proposed to be shorter and more tightly folded. Thus, the scaffold stands up to its assigned organizational role: it is more tightly coiled in the gene-poor Q-regions while it is more uncoiled in the gene-rich R-regions.

Our current model of chromosome structure encompasses previous observations made with more unfolded chromosomes including the notion of mirror symmetry of sister chromatids [24,25]. Thus the extended loop/scaffold model permits a satisfactory description of the morphological aspects of chromosomes. Yet the model falls short of providing a molecular explanation for the process of chromosome condensation.

**SARs are cis elements of chromosome dynamics**

We used the Xenopus chromosome assembly system to ask, are SARs cis elements of chromosome dynamics? Two experimental possibilities were explored to study this question: first, interference by competition with added SAR or non-SAR DNA, or second, inhibition with artificial, super SAR-binding proteins. To explore the first possibility, we studied the conversion of added nuclei to mitotic
The chromatin is quite uniformly stuffed with DNA, but it contains an internal subregion called the AT-queue (yellow) that is generated by juxtapositioning of the SARs as mediated by the scaffold. The AT-queue proceeds from telomere to telomere on an irregular coil-like path [21]. The AT-queue is relatively tightly folded (or coil-like) in regions that are called Q-bands by the cytogeneticist. The AT-queue in the R-band regions is more unfolded and centrally located. The R-band construction can be schematized by the simplest version of the loop model, a central scaffold surrounded by peripheral loops [fig. 3C] [5]. The classic chromosomal banding pattern arises from the irregular folding path of the AT-queue. That is, Q-bands appear AT-richer, in the model yellow-richer, than the R-bands due to the tighter folding of the AT-queue in these regions. Conversely, R-bands appear GC-richer, greener in the model, than Q-bands for the opposite reason. Loops are not directly observed by fluorescence microscopy but inferred and the structural relationship of neighboring loops and their packaging mode are not known.

Sister chromatids relate to each other by mirror symmetry. This conclusion is based on optically sectioned, partially-unfolded human chromosomes immunostained for topoisomerase II. The top and bottom optical sections reveal the opposite handedness of the scaffold coil.

Chromosomes in Xenopus egg extracts in the presence of an increasing concentration of SAR or non-SAR competitor fragments. Although SAR DNA appeared to be a more potent inhibitor of chromosome assembly, as compared to non-SAR controls (about a factor of two), we remained unconvinced by this difference [Adachi, Y., unpublished]. In contrast, inhibition by a super SAR-binding protein was highly successful. These experiments identified SARs as cis elements of chromosome condensation [26].

Fig. 4 Comprehensive chromosome model.
A) The chromatid is quite uniformly stuffed with DNA, but it contains an internal subregion called the AT-queue (yellow) that is generated by juxtapositioning of the SARs as mediated by the scaffold. The AT-queue proceeds from telomere to telomere on an irregular coil-like path [21]. The AT-queue is relatively tightly folded (or coil-like) in regions that are called Q-bands by the cytogeneticist. The AT-queue in the R-band regions is more unfolded and centrally located. The R-band construction can be schematized by the simplest version of the loop model, a central scaffold surrounded by peripheral loops [fig. 3C] [5]. The classic chromosomal banding pattern arises from the irregular folding path of the AT-queue. That is, Q-bands appear AT-richer, in the model yellow-richer, than the R-bands due to the tighter folding of the AT-queue in these regions. Conversely, R-bands appear GC-richer, greener in the model, than Q-bands for the opposite reason. Loops are not directly observed by fluorescence microscopy but inferred and the structural relationship of neighboring loops and their packaging mode are not known.

B) Sister chromatids relate to each other by mirror symmetry. This conclusion is based on optically sectioned, partially-unfolded human chromosomes immunostained for topoisomerase II. The top and bottom optical sections reveal the opposite handedness of the scaffold coil.
As potential SAR repressors, we synthesized proteins containing reiterated, AT-hook peptide motifs linked with a spacer of about 25 amino acids [fig. 5]. AT-hook motifs are peptides known to preferentially bind A-tracks through minor groove contacts [27]. We call these proteins MATH for multi-AT-hook; they are expected to bind SARs that are composed of clustered A-tracks with extreme specificity. In contrast to SARs, they should bind poorly to regions containing isolated AT-tracks, since the latter, as opposed to SARs, cannot favorably accommodate the numerous linked AT-hooks of the MATH-protein [fig. 5]. Indeed, our experimental studies demonstrated convincingly that MATH-proteins containing 10 to 20 hooks bind SAR DNA as well as SAR chromatin with exquisite specificity. The binding constant for the best, MATH20, is in the picomolar range.

Targeting SARs: MATH-Proteins

MATH: Multi-AT-hook protein

AT-hook motif

Strong interaction

DNA

SAR
Clustered A-tracks

Weak interaction

Single A-tract

Fig. 5 MATH-proteins: synthetic super SAR-binding proteins. SARs are DNA elements that define in vitro the bases of the chromatin loops; their specificity is due to numerous A-tracks whose non-B DNA structure is preferentially recognized by SAR-binding proteins such as topoisomerase II. To study the role of SARs in chromosome dynamics, we made artificial super SAR-binding proteins consisting of reiterated AT-hooks linked by a flexible peptide spacer. AT-hooks are peptide motifs derived from the HMG-1/2 protein that preferentially interact with A-tracks through the minor groove [27]. These proteins are called MATH for multi-AT-hook. Such molecules are expected to interact with SARs that are composed of clustered A-tracks with great specificity, since SARs can favorably accommodate the numerous linked AT-hooks of the MATH-proteins. In contrast, they would bind poorly to regions with isolated A-tracks. Indeed, MATH-proteins demonstrably bind SAR DNA and SAR chromatin with remarkable specificity; they are potent inhibitors of chromosome assembly [26]. We plan to synthesize MATH-like drugs with the goal of interfering with neoplastic growth.
Specific inhibitors of chromosome assembly. Xenopus extracts assemble mitotic chromosomes from input sperm nuclei through a number of morphological intermediates called "grapes" and "ruffles" as depicted. The mitotic structures are single chromatids often arranged in clusters, since sperm nuclei are haploid. During this assembly process, a number of scaffolding proteins such as topoisomerase II and XCAP C&E are incorporated into the assembled chromosomes. These biochemical events are not perturbed by addition of the MATH-protein repressors. In contrast, MATH20 (20 hooks) blocks the process of converting the early intermediates to rod-shaped chromatids. Since we demonstrated directly that these repressors are specifically bound to SARs, we conclude that SARs are cis elements implicated in shape-determination of chromosomes. Remarkably, SARs are also involved in the structural maintenance of chromosomes since late addition of MATH20, once chromatids have formed, results in their collapse to spherical chromatid balls. This collapse is thought to be due to a dissociation of the scaffold interactions which maintain chromosomal structure.
Assembly of metaphase chromosomes from added sperm or somatic nuclei by *Xenopus* egg extracts goes through a number of intermediates to form individual chromatids. We used this system to study the role of SARs in chromosome assembly. The biological activity of the MATH-proteins is stunning, they are highly specific inhibitors of the conversion of the nuclear chromatin mass into rod-shaped chromatids. MATH-proteins do this by inhibiting shape-determination rather than chromatin condensation. In their presence, chromatids do not form, but morphologically aberrant structures accumulate that have a mitotic-like extent of compaction. The morphology of these products depends on whether sperm or somatic nuclei are used as substrates [fig. 6]. MATH-proteins also do not affect early mitotic events. Thus, we noted that normal biochemical remodeling of sperm nuclei and the subsequent incorporation of the scaffold proteins (topo II and XCAP C&E) appeared unaffected by MATH. In case of somatic nuclei, the breakdown of the nuclear membrane-lamina complex and the disassembly of the nucleolus was unaffected by MATH.

Interestingly, the MATH-inhibitors not only block shape-determination, they also interfere with the structural stability of chromatids. Addition of MATH to formed chromatids results in an ATP and extract-dependent collapse of the chromatids into individual spherical balls, apparently due to a collapse of the scaffold. In conclusion, the MATH-inhibitors interfere both with the structural maintenance and the shape-determination of chromatids.

Stoichiometric considerations concerning these inhibitors are impressive. By way of example, the best inhibitor MATH20, which contains 20 AT-hooks, severely blocks assembly at a dose of one nanogram. In contrast, no inhibition is observed with 1200 nanograms of the natural HMG-I/Y protein which contains three AT-hooks. Under our experimental conditions, one nanogram of MATH20 represents a single molecule bound per 15 kb of DNA. Besides these stoichiometric considerations, we directly demonstrated the specific association of MATH-proteins with SARs. These observations convinced us that SARs are targets of the inhibitors. Consequently, SARs are cis elements of chromosome dynamics implicated in chromosome shape-determination and shape maintenance.

**Perspective**

Using the novel approach of constructing for the first time artificial inhibitors composed of reiterated DNA-binding motifs targeted to fuzzy sub-elements, we have functionally defined a DNA element involved in chromosome structure. Interestingly, this approach could be employed to target DNA-binding drugs. Many DNA-binding drugs with antiviral and antitumor activity, such as distamycin, bind DNA with a sequence preference. Yet this preference is not high enough to allow precise targeting to essential DNA elements. We propose that specific targeting to an essential, fuzzy DNA element could be achieved by the approach described above, namely by covalently linking drugs such as AT-hooks with an appropriate spacer either in a serial or parallel arrangement. Because of their specificity, such MATH-like drugs are expected to be biologically active at much lower concentrations and also possibly to reduce unwanted side-effects.

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