# Chromosomal loop anchorage sites appear to be evolutionarily conserved

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We have previously identified a class of DNA sequence elements, termed matrix association regions (MARs), which specifically bind to nuclear matrices in vitro and are believed to be at the bases of chromosomal loops in vivo [1]. Here we demonstrate that nuclear matrices prepared from the yeast *Saccharomyces cerevisiae* will specifically bind an MAR sequence derived from the mouse kappa light chain immunoglobulin gene. This suggests that both MAR sequences and their binding sites have been strongly evolutionarily conserved.

*Nuclear matrix* *Nuclear scaffold* *Chromatin loop* *Immunoglobulin gene* *Enhancer* *Topoisomerase II* *(Yeast)*

## 1. INTRODUCTION

DNA loops within the interphase nucleus appear to be anchored to the matrix by an evolutionarily conserved class of (A+T)-rich sequences [1]. These matrix association regions (MARs) appear to contain consensus sequences for topoisomerase II [1,3]. This enzyme is enriched in nuclear matrix preparations [4] and appears to be at the base of mitotic chromosomal loops [5–7]. It is also believed that torsional stress is introduced into DNA during transcriptional activation [8,9] and so MARs may function as both chromosomal loop anchor points and sites of gyration. Particularly intriguing is the observation that MARs occur adjacent to the enhancer elements of both the immunoglobulin light [1] and heavy chain genes (Cockerill and Garrard, in preparation). Here we demonstrate that an MAR located in the mouse kappa light chain immunoglobulin gene binds equally well to nuclear matrices prepared from either mouse or yeast. This together with previous results strengthens the view that MAR sequence binding sites are evolutionarily conserved.

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## 2. MATERIALS AND METHODS

Nuclear matrices were prepared from the mouse plasmacytoma cell line MPC-11 as described [1]. Nuclei were isolated from *Saccharomyces cerevisiae* as in [10] and nuclear matrices were prepared by minor modifications of [11] (Allen and Douglas, unpublished). In vitro DNA-binding assays employed approx. $1.6 \times 10^7$ MPC-11 matrices or $7 \times 10^8$ yeast matrices in a 100 $\mu$l volume in the presence of 100 $\mu$g/ml sonicated *E. coli* DNA, as in [1]. Input and bound purified DNA samples were resolved by gel electrophoresis and bands were visualized by autoradiography [1]. The yeast nuclear matrices were kindly provided by Jerry Allen of this Department.

## 3. RESULTS AND DISCUSSION

MARs appear to be an evolutionarily conserved class of chromosomal loop anchorage elements.

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The mouse kappa light chain immunoglobulin gene possesses a 365 bp MAR just upstream of the enhancer and the analogous rabbit gene appears to contain a similarly positioned MAR [1]. The enhancer of the mouse heavy chain immunoglobulin gene is flanked by a pair of MARs (Cockerill and Garrard, in preparation). Also, we have previously shown that a *Drosophila* histone gene MAR will specifically bind to mouse nuclear matrices, and compete for the same binding sites recognized by mouse MAR sequences. These binding sites are abundant and so far do not appear to be tissue-specific [1].

To study further the evolutionary conservation of MAR sequence binding sites we have used an in vitro assay to compare the binding of the mouse kappa light chain immunoglobulin gene MAR sequence to mouse and yeast nuclear matrices. The probe used was an end-labeled *Bam*HI-*Hin*dIII digest of the recombinant plasmid pG19/45 [1], which yields a 2.85 kb kappa gene fragment and a 4.0 kb pBR322 fragment that serves as a control for non-specific binding. As shown in fig.1A, the 2.85 kb kappa gene fragment that contains the MAR preferentially binds to both mouse and yeast nuclear matrices.

To demonstrate that the kappa gene MAR is indeed responsible for the observed binding to yeast nuclear matrices we studied the binding of *Ava*II-digested pG19/45. As shown in fig.1B, only a 999 bp fragment that contains the MAR is significantly bound by either mouse or yeast nuclear matrices. This fragment contains only 335 bp of the kappa gene region corresponding to the MAR and 664 bp of pBR322 [1] (fig.1C).

Thus, we show here that a mouse MAR sequence binds specifically to yeast nuclear matrices. Previously we demonstrated that a *Drosophila* MAR sequence binds specifically to mouse nuclear matrices [1]. In other studies on the human $\beta$-globin gene we have found regions upstream and within the gene that preferentially bind to mouse plasmacytoma cell nuclear matrices (Cockerill and Garrard, unpublished) and in an earlier study [12] we identified an MAR within the SV40 genome that binds to matrices from a variety of mouse cell types. It therefore appears that MARs and their binding sites have been evolutionarily conserved.

The finding of MAR sequence binding sites in yeast should permit more detailed biochemical and genetic studies in a well-defined system. In particular, a series of *cis*-acting DNA sequences have been elucidated in yeast, which include upstream activators, promoters, terminators, replication origins, centromeres, telomeres and an element that stabilizes plasmid segregation (see [13–16]). Which of these sequences bind specifically to yeast nuclear matrices remains to be determined. Furthermore, it should be possible to clone the genes that specify and regulate MAR sequence binding sites. Disruption and analysis of these genes may provide considerable insight into the biological significance of the higher order organization of genes in living cells.

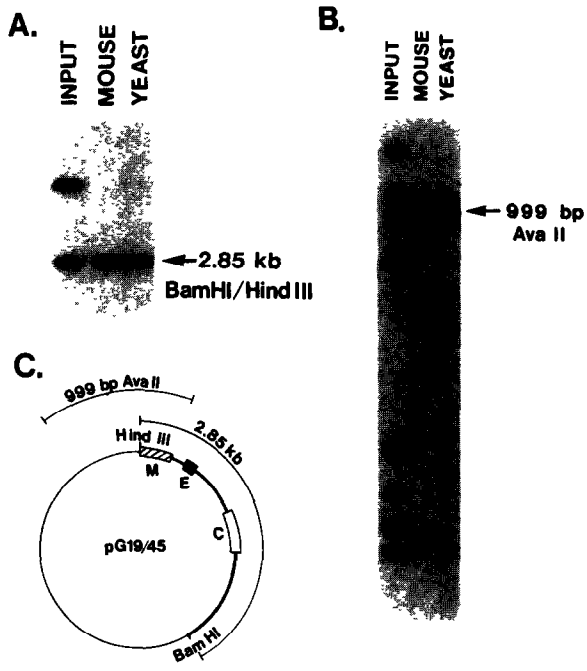


Fig.1. In vitro DNA-binding assay of the mouse kappa light chain immunoglobulin gene MAR to mouse and yeast nuclear matrices. (A) Binding of *Bam*HI-*Hin*dIII digested pG19/45 to mouse plasmacytoma and *Saccharomyces cerevisiae* nuclear matrices. (B) Binding of *Ava*II-digested pG19/45 to the above mouse and yeast nuclear matrices. (C) Map of pG19/45 [1] showing the 2.85 kb *Bam*HI-*Hin*dIII fragment of the mouse kappa light chain immunoglobulin gene (heavy line) inserted in pBR322. Indicated by boxes are the MAR (M), enhancer (E) and constant region exon (C). Fragments preferentially bound by nuclear matrices are indicated.

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