The Enhancer of the Immunoglobulin Heavy Chain Locus Is Flanked by Presumptive Chromosomal Loop Anchorage Elements

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We have located presumptive chromosomal loop anchorage elements within the mouse heavy chain immunoglobulin locus. Analysis of 31 kilobases spanning diversity, joining, enhancer, switch, and the \( \mu \) and \( \delta \) constant regions reveals that only a single 1-kilobase segment exhibits specific binding to nuclear matrices. It is of particular significance that the transcriptional enhancer element resides within this matrix association region (MAR). Fine structure mapping indicates that binding is mediated by A+T-rich ~350-base pair segments that reside on either side of the enhancer. The MAR sequences residing 5' of the enhancer contain topoisomerase II consensus sequences like the MAR located upstream of the \( \kappa \) light chain gene enhancer. The heavy chain gene MARs, however, exhibit a lower affinity for matrix association compared to the \( \kappa \) gene MAR. Significantly, the juxtaposition of enhancer elements with MARs appears to be evolutionarily conserved within the immunoglobulin genes, suggesting that MARs may act as positive and/or negative regulators of enhancer function.

DNA within interphase nuclei and mitotic chromosomes is organized into topologically constrained looped domains of about 10–100 kilobases in length (1-4). Given the chemical complexity of the mammalian genome, one can estimate that roughly 10\(^6\) chromosomal loop anchorage sites would exist in a single diploid nucleus. With these considerations in mind, a number of interesting questions can be entertained. Do anchorage sites punctuate gene clusters into functionally distinct chromatin domains? Do such sites target genes in the nucleus to specific compartments? Are common anchorage elements shared by functionally diverse genes or only by related gene families? Finally, are different regions of a given gene anchored depending on transcription, replication, development, or tissue type?

To search for presumptive chromosomal loop anchorage elements, in an earlier study we developed an in vitro DNA binding assay that localizes matrix association regions (MARs)\(^*\) within cloned genes (5). This approach can be complemented by the nuclear halo mapping procedure of Laemmli and co-workers (6), which employs nuclear fractionation of endogeneous sequences to identify scaffold attached regions (SARs) (7). A limited comparison between these two procedures reveals that SARs appear to be strictly analogous to MARs ((5, 7) wide infra). Interestingly, SARs are located nonrandomly and at specific sites adjacent to a series of functionally distinct Drosophila class II genes (6-9). Available evidence suggests that the positions of these contact points appear not to vary with gene expression, but technical limitations preclude a definitive conclusion that anchorage is constitutive (7). Furthermore, studies on the \( \kappa \) light chain immunoglobulin gene in mouse cells reveal a MAR adjacent to the transcriptional enhancer element that is in the same position both before and after recombination and gene expression (5). In addition, certain SARs of Drosophila genes also appear to be located near enhancer-like elements (7). Significantly, Drosophila gene SARs share a number of features in common with the mouse \( \kappa \) gene MAR, including overall A+T richness, the presence of topoisomerase II consensus sequences, short clusters of certain characteristic A+T-rich sequences, and the ability to compete for specific binding sites in mouse nuclear matrix preparations (5-9). The presence of topoisomerase II sites is interesting, considering that this protein is the major component of the mitotic chromosome scaffold (10-12) and is recovered in high yield in certain nuclear matrix preparations (13). Whether topoisomerase II is responsible for anchoring these sequences at the base of chromosomal loops in the interphase nucleus in vivo, however, remains to be demonstrated.

The juxtaposition of the \( \kappa \) gene enhancer with a MAR that contains topoisomerase II sites suggests that a functional relationship may exist between transcriptional enhancement and DNA swirling at the base of chromosomal loops (5). Consistent with this view is the apparent in vivo localization of topoisomerase II adjacent to the SV40 enhancer (14) and the in vitro activation of dynamic supercoiling of the Xenopus 5 S gene by trans-acting factors (15). In the present study we address these issues further by an analysis of the mouse heavy chain immunoglobulin locus. Previous studies have identified a powerful tissue-specific enhancer (E\(\kappa\)) in the intron between the joining (\( J\)) and switch recombination sequences (S) just upstream of \( \mu \) constant region exons (C\(\mu\)) (16, 17) (for review of immunoglobulin genes, see Ref. 18). We show here that this enhancer is flanked on both sides by presumptive chromosomal loop anchorage elements. The 5' MAR appears similar to the \( \kappa \) gene MAR as it contains topoisomerase II sites. Therefore, the heavy and light chain mouse immunoglobulin genes have a strikingly similar DNA sequence organization with respect to MARs and enhancers.

EXPERIMENTAL PROCEDURES AND RESULTS AND DISCUSSION

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Matrix Association Regions within the Heavy Chain Locus

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Matrix Association Regions within the Heavy Chain Locus

The Heavy Chain Gene Enhancer is Flanked by MNHs. To address whether enhancer activity is flanking sequences (of both) contribute to activity enhancement, we mapped binding sites further. Fig. 2A shows that 79% and 43% of the $5'$ end fragments of 280 bp each were bound by the enhancer. The binding of a 784 bp fragment that contains 68 bp of $3'$ enhancer sequence is also shown. Fig. 2B, 2C, and 2D. The map shown in Fig. 2B indicates the enhancer site for the 280 bp core region of the heavy chain gene enhancer that has been delineated by other investigations (31, 32, 33). The 531 bp of Fig. 2C fragment resides 3' of the enhancer core, while the 392 bp fragment encompasses both the enhancer and 5' flanking sequences. The 392 bp fragment was further divided by 5% from the region that covers with the enhancer site. The enhancer site is indicated by dotted vertical lines and the cross-hatched box.

Liver

Heavy Chain Gene MNHs and the Association of Nuclear Heterochromatin. The results of this study show that the heavy chain gene MNHs with chromosomal loop bases in nuclear halos in less pronounced than that of the kappa genes. Furthermore, the 392 bp MNH is more firmly attached to the nuclear halos in nuclear halos with the 5' MNHs than that of the kappa genes, thereby suggesting that the MNHs binding activities (Fig. 2B) is possible that experimental manipulations may differentially disrupt these different contacts and access for the observed variations both between cell sources and expression of MNH-containing elements.

FIG. 3A. Mapping heavy chain gene enhancer in nuclear halos. (A) Nuclear isolated from the spleen were extended with 35S and labeled with MNHs for northern analysis, employing probes. A radioactive placental placenta digoxin and exhibited. Panel A shows a map of the genomic region that the genomic data of Fig. 1. The arrows and designated restriction fragments indicate 5' and 3' MNH-containing elements.

FIG. 2. Binding of the heavy chain gene enhancer to different nuclear regions. Nuclear matrix preparations from the indicated sources (5', 6') or MNHs were hybridized to a 280 bp continuous sequence (P.Y. Tucker and F.R. Blattnar, unpublished results). The map shown in Fig. 2B indicates the enhancer site for the 280 bp core region of the heavy chain gene enhancer that has been delineated by other investigations (31, 32, 33). The 531 bp of Fig. 2C fragment resides 3' of the enhancer core, while the 392 bp fragment encompasses both the enhancer and 5' flanking sequences. The 392 bp fragment was further divided by 5% from the region that covers with the enhancer site. The enhancer site is indicated by dotted vertical lines and the cross-hatched box. The position of the MNH-containing restriction fragments are shown relative to the map of pBR 322, 5' and 3' MNHs are depicted by cross-hatched triangles, and regions of 3' and 5' MNHs are also indicated. Maps do not include vector sequences.
Matrix Association Regions within the Heavy Chain Locus

DISCUSSION

Presumptive Anchor Site(s) Adjacent to the Immunoglobulin Gene Enhancers Appear to Be Evolutionarily Conserved. We demonstrate here that the enhancer region of the heavy chain immunoglobulin gene is located between presumptive chromosome loops 4' and 3.5 (Fig. 4). The 3.5 DNA also contains a DNA adjacent to the enhancer (3.5). Furthermore, the juxtaposition of the DNA with the immunoglobulin gene enhancers appears to be evolutionarily conserved. Both these elements share sequence similarities (Fig. 3). One hypothesis is that regions corresponding to the 3.5 DNA (3.5) have evolved to be conserved with some elements of the heavy chain genes. This evolutionary conservation suggests that the DNA sequences serve crucial roles in the functional organization of the immunoglobulin genes.

Significance of Positioning DNA Adjacent to Enhancers. The presence of DNA next to the immunoglobulin gene enhancers strongly suggests that a functional relationship exists between these two classes of sequences. Previous studies have shown that these enhancers act over considerable distances and independently of the presence of intervening promoters. Thus, the presence of DNA next to these enhancers suggests that they may be involved in transcriptional regulation. The presence of DNA next to the enhancers also suggests that they may be involved in the negative regulation of transcription. This may be especially important in the case of the enhancer since it is located in the regulatory region of the heavy chain genes.

![FIG. 4: Competition between Immunoglobulin Light and Heavy Chain Gene Enhancers Reveals That the E, Gene Enhancer Has Higher Affinity.](image)

The heavy chain enhancer and DNA are located in a DNA-binding assay with 32P-labeled restriction fragments derived from DNA 5': E4/3 enhancer and DNA (see Fig. 5). Autoradiographs are shown of a 32P acrylamide-0.1% DNA gel containing electrophoretically resolved DNA fragments purified from input and matrix-bound fractions. The 5', 3', and 3' DNA concentrations used in the binding experiments are also shown. The arrows designate the DNA fragments that contain the E, enhancer and DNA. The results of the competition assay revealed that the heavy chain enhancer binds to E, DNA with higher affinity than the light chain enhancer. This suggests that the heavy chain enhancer is a more potent regulator of transcription than the light chain enhancer.

The immunoglobulin gene enhancers are also subject to negative regulation (reviewed in Ref. 31), and DNA and RNA could play roles in these processes. If the use of DNA for negative regulation is regulated, and has gone undetected because of artificial binding to nuclear DNA sequences, then one might imagine that the establishment of the attachment could block the access of transcription factors or the spreading of conformational changes in chromatin from the enhancer to the promoter. Thus, these DNA fragments may be involved in the negative regulation of transcription. In this context, we identify here as having negative regulatory effects on gene expression (53, 38, 39). These effects are mediated by the presence of the enhancer DNA. The results presented here emphasize the potential for both positive and negative regulation of gene expression. Clearly, from this discussion of potential DNA function, it is apparent that we need some understanding of the regulatory mechanisms that control transcription. Understanding these mechanisms may help to clarify the role of DNA in transcriptional regulation.

![Complexity of DNA Sequences and Binding Sites.](image)

Our studies together with those of Loomis and co-workers reveal that DNA and RNA are always of sign relevance to DNA-DNA interactions with DNA, to DNA-DNA interactions with RNA, and to DNA-RNA interactions with DNA. The interactions between DNA and RNA are complex and may involve the formation of secondary structures. Furthermore, interactions between DNA and RNA may be influenced by the presence or absence of binding sites. Taken together, these results suggest that while chromosomal loops may be associated with these interactions, additional processing may be required for anchoring loops, particularly in the interphase nucleus.