Murine Laminin B1 Gene Regulation during the Retinoic Acid- and Dibutylryl Cyclic AMP-induced Differentiation of Embryonic F9 Teratocarcinoma Stem Cells*

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Retinoic acid (RA) and cyclic AMP analogs cause the differentiation of F9 embryonic teratocarcinoma stem cells into parietal endoderm, an epithelial cell of the early mouse embryo. Laminin B1 is induced in this differentiation process, but is not transcriptionally activated until 24–48 h after RA addition and is not maximally induced until approximately 72 h. Cyclic AMP analogs enhance this transcriptional activation. Although several DNase I hypersensitive sites (DHSS) were observed in the LAMB1 5′-flanking DNA, one of the sites, DHSS2, was detected only after 72 h of RA treatment. Transient transfections have demonstrated that the DHSS2 region functions as a “late-acting RA-inducible enhancer,” and motifs in this enhancer contain the homeobox protein-binding site TTATTAACA. Greater enhancer activity concerning the structure and functions of laminins has been demonstrated recently; in this nomenclature, the laminin B1 protein is called β1 and the gene is LAMB1.

From sequencing clones from an EHS expression library, the cDNA sequences of the murine laminin B1a chain (18), the B2a chain (20), and the Ae chain (21) have been determined. The sequences of human laminin B1 (22, 23), B2 (14, 24), and the A chain (25, 26) have also been determined.

Laminins are glycoproteins which are constituents of a particular type of extracellular matrix called the basement membrane; basement membranes are synthesized by epithelial, endothelial, nerve, muscle, and other cell types. Laminins have a number of functions in the processes of cell adhesion, cell migration, proliferation, differentiation, neurite outgrowth, and tumor metastasis (for review, see Ref. 2). Much information concerning the structure and functions of laminins has come from the mouse Engelbreth-Holm Swarm (EHS) tumor (3). Three protein chains are constituents of EHS laminin, Ae (M, = 400,000), B1a (M, = 215,000), and B2e (M, = 205,000). These three polypeptide chains are assembled into a cross-like structure with a molecular weight of approximately 940,000 (4, 5). Recently, additional laminin chains related to Ae (Am; Refs. 6 and 7) or B1e (B1s; Ref. 8) have been reported. The human A chain homolog Am has also been named merosin (6) and is the same as the chain found in heart (7). A specific deficiency in the laminin Am chain has been associated with the dystrophic dy mutation in mice (9, 10). The dy mutation represents a severe neuromuscular disease resembling human muscular dystrophy. Furthermore, other laminin-related proteins have been reported (11–13). A human variant B2 chain named laminin B2t has been described (14). An epithelial specific laminin chain called laminin B1k has also been reported (15). Various heterotrimers can be assembled from the different laminin subunits (16). The nomenclature used is that of Engel et al. (17). Another nomenclature for the protein chains has also been proposed recently; in this nomenclature, the laminin B1 protein is called B1 and the gene is LAMB1.

During early mouse embryogenesis, mRNAs for the B1 and B2 chains of laminin were detected from the 4-cell stage, while the A chain appeared at the 16-cell stage (27, 28). During the period of mouse development in which major organs are forming, laminin was detected in the basement membranes of epithelial cells (29), during intestinal development (30), and in ureteric buds and nephrogenic vesicles during early prenatal kidney development (31). The conversion of mesenchyme to epithelium in kidney is accompanied first by an increase in laminin B1 and B2 transcripts (31, 32) and is followed by an increase in A chain expression (32). The α1β1 and α6β1 integrin heterodimers have been shown to mediate cell attachment to distinct sites on laminin (33). The α6β1 laminin receptor is also regulated during development both by its level of expression and via phosphorylation (34–36).

The expression of laminin has been studied in a number of model cell culture systems. In cultured neuroepithelial cells, basic fibroblast growth factor enhances the amount of laminin expressed at the protein level (37). During myogenic differentiation, the expression of several different laminin chains is increased (38). Retinoic acid, a member of the vitamin A family of signaling molecules called retinoids, induces laminin expression in cultured murine embryonic teratocarcinoma stem cells such as the F9 cell line which is induced to differentiate into parietal endoderm cells (39). The levels of transcripts encoding the laminin A, B1, and B2 polypeptide chains are increased in F9 cells treated with RA1 as compared to undifferentiated stem

1 The abbreviations used are: RA, all-trans-retinoic acid; RARE, retinoic acid response element; EMSA, electrophoretic mobility shift assays; RACT, retinoic acid, dibutylryl cyclic AMP, and theophylline; LAMB1, laminin B1 gene; DHSS, DNase I hypersensitive site(s); bp,
cells (40–45). The laminin B1 chain produced by RA-treated F9 cells appears to be identical with the murine B1e chain as determined by DNA sequencing of partial cDNA clones.²

Further studies by this laboratory demonstrated that the RA-mediated regulation of the expression of the LAMB1 gene occurred primarily at the transcriptional level (46, 47). Cyclic AMP analogs were shown to augment the transcriptional response of the cells to retinoic acid, but cyclic AMP analogs alone did not enhance the rate of transcription of this gene (44, 46, 47). An RARE (retinoic acid response element) was identified at position −477 to −432 in the promoter region of the murine LAMB1 gene, and this response element is recognized by retinoic acid receptors (RARs) (48, 49). Furthermore, the targeted disruption of both copies of the RARγ gene in F9 cells led to a defect in the RA-associated activation of the LAMB1 gene (1). This suggested that the LAMB1 gene was a target gene regulated by the RARγ. However, the late induction of the LAMB1 gene and the observation that the RA-mediated induction was prevented by the protein synthesis inhibitor cycloheximide (50) indicated that other RA-inducible transcription factors were involved in the regulation of the expression of the LAMB1 gene. In this manuscript, we present a more detailed analysis of the regulation of the LAMB1 gene in response to retinoic acid.

EXPERIMENTAL PROCEDURES

Cell Culture—F9 cells were cultured as previously reported (48). F9 cells were induced to differentiate in the presence of RA or RACT as described previously (51). The F9 RARα−/− and RARγ−/− cell lines were cultured as described by Boylan et al. (1, 52). PYS-2 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5% heat-inactivated fetal calf serum.

Isolation of the Murine LAMB1 Genomic Clones—A λEMBL library (obtained from Dr. Anton Berns) containing murine 129SV genomic DNA was used for the isolation of LAMB1 genomic clones. The screening protocol was that of Sambrook et al. (53). A total of 8 × 10⁷ phage clones were screened by hybridization with a 2.3-kb HindIII/BamHI probe covering from −3961 to −1614 bp of the murine LAMB1 gene; this probe was identified previously in this laboratory (48). Four positive phage clones were further screened and purified as described by Sambrook et al. (53). Clones containing genomic LAMB1 inserts were mapped by a series of restriction enzyme digestions.

Plasmids—The construction of pl−3166−7376TKCAT was accomplished by donating a 400-bp Dral/HindIII murine genomic fragment (Dral converted to HindIII) into pBlCAT2 (54). The plasmids p13LAMCAT, p0.49LAMCAT, and p3.9LAMCAT were described previously (48). The plasmids p2.8LAMCAT, p3.4LAMCAT, and p3.6LAMCAT were constructed by J. D. Gold in this laboratory. The murine RARγ expression plasmid, and pβ-actin-IαZ2 were previously described (48).

Nuclear Protein Preparations, DNase I Footprinting Assays, and Electrophoretic Mobility Shift Assays (EMSA)—Nuclei and nuclear extracts were prepared according to Dignam et al. (55). Footprinting probes were prepared by 5' end labeling of DNA restriction fragments with a Klenow fragment of DNA polymerase and isolated after secondary restriction enzyme digestion (56). Markers were prepared by chemical sequencing reactions (57). DNase I footprinting assays were performed as described by Lichtsteiner et al. (58). The DNase I enzyme (melanoma cell biological grade, from bovine pancreas) was purchased from Worthington Corp. EMSA analysis was performed according to Ausubel et al. (59). The wild type and mutant laminin B1 probes are described in Fig. 6D. A nonspecific oligonucleotide was also used (sequence is written 5' to 3'): GTGAGAACCTAGCCAACGCTCCCTAAATGGC.

Other Procedures—Transient transfections, chloramphenicol acetyltransferase assays, and mapping of DNase I-hypersensitive sites were performed as described previously (48, 60).

RESULTS

Mapping of DNase I Hypersensitive Sites—Prior research had identified an RARE at position −477 to −432 in the murine base pair(s); kb, kilobase(s); CAT, chloramphenicol acetyltransferase; CRE, CAMP response element.

² C. Stoner, J. Gold, and L. Gudas, unpublished observations.

³ C. Li and L. J. Gudas, unpublished data.

![Mapping DHSS of the LAMB1 gene in F9 cells.](http://www.jbc.org/)

**FIG. 1.** Mapping DHSS of the LAMB1 gene in F9 cells. A, the line diagram shows the LAMB1 gene with the positions of the Apal (a), BamHI (b), HindII (h), and PstI (p) sites in the genomic DNA relative to the transcription initiation site and the regions to which the DHSS map. Probes for DHSS mapping are indicated. Probe SA is a 466-bp Sall/Apal fragment covering from −353 to +113 bp of murine LAMB1 gene (hatched box). Probe HS is a 538-bp HindII/SpeI fragment covering from −3961 to −3423 bp of murine LAMB1 gene (open box). B, mapping of distal DHSS 5' to the LAMB1 gene. Nuclei from F9 cells were treated with DNase I (1, 3, and 10 units). DNA from DNase I-treated nuclei was digested with Apal, and the Southern blot was hybridized with the probe SA as described in A. The positions of DHSS are given. The RA concentration was 1 × 10⁻⁶ M. This experiment was performed three times, and similar results were obtained in all three experiments.
observed in DNA from nuclei treated with RA for both 24 and 72 h, reflecting an RA-inducible hypersensitive site (Fig. 1, A and B). DNA from the 72-h RA-treated F9 cells, but not from the 24-h RA-treated F9 cells, was cleaved at an additional site that is located approximately −2.9 kb from the transcription initiation site, resulting in an additional band of approximately 3 kb (DHSS2) (Fig. 1, A and B). Similar DHSSs were observed in nuclei from F9 cells cultured in the presence of RACT (1 × 10−6 M RA plus 250 μM dibutylryl cyclic AMP and 500 μM theophylline (data not shown)).

To identify any DHSS closer to the initiation of transcription of the LAMB1 gene, DNA from DNase I-treated nuclei was digested with HindIII, analyzed by gel electrophoresis, and the resulting Southern blot was hybridized to a 538-bp HindIII/Sph fragment containing −3961 to −3423 bp of mouse LAMB1 gene (Fig. 1A). Digestion of DNA in nuclei from untreated F9 stem cells resulted in a band of 3.7 kb (DHSS1) that maps to about −260 bp from the transcription initiation site. While this site, DHSS1, is located relative close to the LAMB1 RARE (48), this site was observed only in nuclei from stem cells and was not observed in DNA from nuclei of 24-h and 72-h RA-treated F9 cells (data not shown).

Overall, we have examined a total of 18 kb of genomic DNA at the 5′ end of the murine LAMB1 gene. Three RA-sensitive DHSSs were observed. DHSS1 is detected in F9 stem cells but is not observed after 24 h or 72 h of RA treatment. DHSS2 is observed only after 72 h of culture in the presence of RA, while DHSS3 is observed after both 24 h and 72 h of RA treatment. Because the DHSS2 was observed only at later times after RA addition (72 h), we concentrated on the further delineation of this site since its properties reflected the pattern of expression of the endogenous LAMB1 gene.

Isolation of the Murine LAMB1 Genomic Clones and Analysis of the DHSS2 Region of the Murine LAMB1 Gene—Four phage clones that contained mouse LAMB1 genomic DNA were isolated by screening a mouse genomic library with a 2.3-kb HindIII/BamHI probe covering from −3961 to −1614 bp of the LAMB1 gene. From a total of 8 × 10⁵ phage clones that were screened, 4 phage clones resulted in positive hybridization signals.

In order to define the regulatory elements in the DHSS2 region of the murine LAMB1 gene in greater detail, the series of LAMB1 promoter/CAT constructs indicated in Fig. 2A were transfected into F9 cells, and this transfection was followed by treatment of the cells with RA for 24 h. For the 72-h time point, the cells were cultured in the presence of RA for 48 h, and then the cells were transiently transfected. This transfection was followed by further culture in the presence of RA for an additional 24 h. The results of these transient transfection assays indicated that all of the LAMB1 promoter/CAT constructs except the p0.13LAMCAT construct exhibited an increase in activity after 24 h of RA treatment (Fig. 2B). These results confirmed previous work by Vasios et al. (48, 49) which demonstrated that the p0.49LAMCAT construct which contains the RARE was RA-responsive by 24 h after RA addition.

Further increases in CAT activity were observed after 72 h of RA treatment for most of the LAMB1/CAT constructs (Fig. 2B). Deletion of a 569-bp fragment containing the DHSS2 from the 5′ end of p3.4LAMCAT resulted in much less additional CAT activity at 72 h versus 24 h (Fig. 2B). Taken together, the DNase I hypersensitivity data indicating the location of DHSS2 at about −2.9 kb, and the results of these transient transfection assays are consistent with the hypothesis that the region of the LAMB1 promoter between −2.8 kb and −3.4 kb contains a DNA regulatory site which is more active at later times (72 h) after RA treatment than at 24 h.

Footprinting Studies—To determine whether the DHSS2 region could be recognized specifically by nuclear proteins, in vitro footprinting studies were performed with various subfragments of the 687-bp fragment covering the DHSS2 region. Three protected regions (FP2892, FP2860, and FP2821) were observed with the extract of 72-h RA-treated F9 cells (Fig. 3A). The footprint locations and sequences are shown in the diagram in Fig. 3B. Thus, specific regions of the LAMB1 promoter could be footprinted, but the footprinting data did not indicate any differences between RA 24-h extracts (data not shown) and RA 72-h extracts. Since EMSA is more sensitive and quantitative, we next examined this region of the LAMB1 gene using EMSA.

Characterization of Nuclear Protein Binding by EMSA—To characterize further individual protein-binding sites within the footprinted regions, EMSA was performed with double-stranded, labeled oligonucleotides corresponding to P2892, P2860, and P2821. With extracts from untreated F9 stem cells, two retarded bands, complexes C1 and C3, were observed when probe P2860 was tested (Fig. 4A). In extracts from F9 cells treated for 24 h with RA, both retarded complexes C1 and C3 were again observed. Moreover, a small amount of a new complex C2 was detected at 24 h. In extracts from cells treated with RA for 72 h, a large amount of the complex C2 was observed, whereas the C1 complex was absent (Fig. 4A). Strikingly, when...
extracts from cells treated with RACT were examined, the C2 retarded complex was very abundant in extracts from both the earlier time point, 24 h, and the 72-h time point (Fig. 4A). Similar enhancement was observed when another cyclic AMP analog, 8-bromocyclic AMP, was used in combination with RA treatment to generate extracts for EMSA (data not shown). Thus, we conclude that the C2 complex represents a protein-DNA complex which is very abundant at 72 h in the presence of RA alone, but which is present at high levels at both 24 and 72 h when extracts are made from cells treated with RACT. The C2 complex may be functionally significant, therefore, since we also see earlier and more intense transcriptional activation of the LAMB1 gene in the presence of RACT than in

**Fig. 3. DNase I footprinting assays.** A, DNase I footprinting analysis with the nuclear extract from F9 cells cultured in the presence of 1 × 10⁻⁶ M RA for 72 h. A probe spanning nucleotides −3136 to −2735 (DraI/MseI fragment) was used. This probe was prepared as described under “Experimental Procedures.” Increasing amounts of protein extract were used, from 1 μg to 40 μg. The lines indicate the footprints observed. The positions of the footprints are also marked. This assay was performed four times with different extracts; similar results were obtained. B, the sequence of the LAMB1 gene from −2898 to −2787 is shown. The footprinted sequences of individual binding elements are indicated by the underlining. The footprinted sequence is written 5' to 3', with the nucleotide numbers shown on the left.

**Fig. 4. EMSA analysis of the nuclear protein-binding sites in F9 cells.** EMSAs were performed with the radiolabeled P2860, P2821 oligonucleotides (10⁵ cpm/0.5 ng). Lane 1, radiolabeled probe alone. Lane 2, 5 μg of the extract from F9 untreated stem cells. 5 μg of extract from F9 cells treated with RA for 24 h (lane 3), RACT for 24 h (lane 4), RA for 72 h (lane 5), and RACT for 72 h (lane 6) was used. The arrows indicate bands of altered mobility representing specific protein-DNA complexes. A, probe P2860; B, probe P2821. The sequences of probes P2860 and P2821 are shown in Fig. 6D. RA, 1 × 10⁻⁶ M; RACT is RA at 1 × 10⁻⁶ M plus dibutyryl cyclic AMP at 250 μM and theophylline at 500 μM. This experiment was performed three times with different extracts; one experiment is shown, but similar results were obtained in all three experiments.
the presence of RA alone (47).

Analysis using the probe P2821 identified a single major retarded complex, C3 (Fig. 4B). The C3 complex is slightly more abundant in extracts from cells treated with RA for 24 h and in extracts treated with RACT for 24 h (Fig. 4B). Thus, while this C3 complex is present in nuclei from RA-treated cells, it is also present at significant levels in extracts from untreated F9 stem cells. From mutation studies described later in the manuscript (Fig. 6), the C3 complex observed with probe P2821 appears to be identical with the C3 complex observed with probe P2860.

Analysis with probe P2892 resulted in some very weak retarded complexes which appeared to be different from the complexes described above (data not shown). Analysis of these complexes was not pursued further.

The Specificity of the Retarded Complexes—To examine the affinities of the proteins for the DNA in the C2 and C3 complexes, competition experiments were performed. The competition experiments presented in Fig. 5, A and B, indicated that complexes C2 and C3 were specific, since their formation was inhibited by the presence of a 100-fold excess of unlabeled P2860, or P2821, respectively (lane 3 versus lane 1, Fig. 5A; lane 3 versus lane 1, Fig. 5B). The specificity of the proteins for these oligonucleotide probes P2860 and P2821 was also demonstrated by the fact that a 100-fold excess of a nonspecific oligonucleotide (see “Experimental Procedures”) failed to compete with either radiolabeled probe for extract (data not shown).

The C3 complex formed with the P2860 probe was inhibited by a 100-fold molar excess of unlabeled P2821, whereas the C2 complex was not affected by a similar excess of P2821 (Fig. 5A). Reciprocal experiments showed that the C3 complex formed with the P2821 radiolabeled probe was inhibited by a 100-fold molar excess of unlabeled P2860 (Fig. 5B). These data suggest that the same protein is present in the complex C3 detected by both probes P2860 and P2821.

The Retarded Complexes in Murine PYS-2 Cells—It is known that PYS-2 cells (epithelial cells from a murine parietal yolk sac tumor) constitutively produce endogenous laminin B1 protein (61). Thus, extract from PYS-2 cells was also tested by EMSA. In PYS-2 extracts analyzed with the P2860 probe, C2 and C3 complexes were observed, but the C1 complex, as expected, was absent (Fig. 6A, lane 2). In addition, a novel complex C4 was observed (Fig. 6).

The Effect of Mutations in P2860 and P2821 on Protein Binding by EMSA—Oligonucleotides containing mutations were used in EMSA experiments to define more precisely the DNA sequences involved in the DNA-protein interactions. Mutation M1 in probe P2860 prevented the formation of the C1, C2, and C3 complexes (Fig. 6A; Fig. 6D for the sequences of the mutated oligomers). Mutation M2 abolished the C1 and C2 complexes, but had no effect on the C3 complex (Fig. 6B; Fig. 6D for the sequence of the mutated oligomer). Mutation M3 resulted in the loss of the C1 and C2 complexes, data similar to that obtained for M2 (data not shown). Mutation M4 had no effect on any of the complexes C1, C2, or C3 (data not shown). Mutations M5 and M6 abolished complex C3, but had no effect on the C1 and C2 complexes. Mutation M7 had no effect on the complexes C1, C2, or C3 (data not shown). This mutation analysis demonstrated that the binding site for C1 and C2 was TTATTAACA. The binding site for C3 was CTGTCATTA.

The Effect of Targeted Disruptions of the RARα and RARγ Genes—Previous publications showed that the targeted disruption of both copies of the RARγ gene in F9 cells led to a defect in the RA-associated activation of the LAMB1 gene. In contrast, the targeted disruption of the RARα gene had no effect on the LAMB1 gene (1, 52). Therefore, the effects of the targeted disruption of the RARα and γ genes on the expression of a pL2–3136/2736TKCAT construct containing the DHSS2 were examined.

In F9 wild type cells, the expression of the parent plasmid pLBCAT2 was not RA-inducible, while pl-L2–3136/–2736TKCAT expression was RA-inducible (Fig. 7). In addition, the level of CAT activity of pL2–3136/–2736TKCAT could be enhanced by RACT as compared to RA treatment (Fig. 7). The targeted disruption of the RARα gene did not inhibit the RA-induced pL

![Image](http://www.jbc.org/Downloadedfrom)
In contrast, the targeted disruption of the RARγ gene abolished the RA-inducible pL-2736TKCAT expression (not shown). These data show that the functional enhancer requires RARγ nuclear signaling. This signaling is likely to be indirect since the DHSS2 region between -2316 and -2736 does not appear to contain an RARE to which RARγ could bind directly. Furthermore, in this experiment, the pL-2316/-2736TKCAT construct displayed activity in the absence of co-transfected RARs.

Extracts from these two cell lines were then tested by EMSA to determine the effects of the loss of RARγ versus RARα on the protein binding to regulatory elements in this 400-bp DHSS2 region of DNA. The targeted disruption of the RARγ gene had no effect on the behavior of the retarded complexes (Fig. 8, compare lanes 2 and 3 with 4 and 5). In contrast, the targeted disruption of the RARγ gene abolished the RA-associated C2 complex formation, but had no effect on the behavior of the C1 or C3 complexes (Fig. 8, A and B). These data strongly suggest that the C2 protein-DNA complex is of functional significance in the positive regulation of the LAMB1 gene in response to RA since this C2 complex does not form in extracts from RARγ2/2 cells in which the LAMB1 gene is not activated by RA.

DISCUSSION

The Molecular Basis of the Delayed Murine LAMB1 Gene Expression during the Differentiation Process of F9 Teratocarcinoma Cells—A number of genes have been shown to undergo increased expression during the differentiation of F9 cells. Transcription of some of these genes (e.g. the Hox a1 gene) is rapidly induced by RA and is independent of new protein synthesis (62). Expression of these early-response genes such as Hox a1 is mediated through the interaction of RARs with cis elements in promoters or enhancers (60). Other genes, such as LAMB1, exhibit increases in mRNA expression at relatively late times after RA treatment (44, 45).

In order to study the regulation of the LAMB1 gene, we...
searched for changes in chromatin structure that occur when the gene is activated. Our studies reveal that one of the DNase I hypersensitive sites, DHSs2, was observed only at late times (72 h) after RA addition (Fig. 1B). Further, functional transient transfection experiments have demonstrated that the DHSs2 region functions as an enhancer (Figs. 2 and 7). Detailed analysis of this enhancer by EMSA has led to the identification of several protein-DNA complexes in this enhancer (Fig. 4, A and B). Among them, the protein complex C2 is most intriguing because it is (a) found only in nuclear extracts of RA-treated F9 cells and is more abundant at late times (72 h) than at early times (24 h) (Fig. 4), (b) detected in extracts from RA-treated F9 wild type cells and F9 RARα−/− cells, but not in RA-treated RARγ−/− cells (Fig. 8), and (c) detected at earlier times and in greater abundance in extracts from RACT-treated cells versus RA-treated cells (Fig. 4A). Thus, our data are consistent with the interpretation that the protein-DNA complex C2 contributes to the activation of the LAMB1 gene during the differentiation process. Our data also explain how a “late” cytokineximide-dependent RA-inducible gene such as LAMB1 can be positively regulated in a delayed fashion in this differentiation model (Fig. 9).

The Role of Cyclic AMP in the RA-induced LAMB1 Gene Expression—A number of cyclic AMP analogs enhance the RA-activated transcription of many late-response genes such as LAMB1 in F9 cells. The enhancement of gene transcription in response to cyclic AMP analogs in combination with RA may require a new transcription factor that is responsive to cAMP. Thus, transcription factors that interact with cyclic AMP response elements (CREs) such as AP-2 or CRE-binding protein may be important in regulating the cyclic AMP responsive promoters in F9 cells (63, 64). We show that cyclic AMP analogs enhance both the RA-induced C2 complex formation in EMSA experiments (Fig. 4) and the RA-inducible pL−3136/−2736TKCAT expression (Fig. 7). Therefore, our data suggest either that a cyclic AMP responsive transcription factor plays a role in the formation of the C2 protein-DNA complex, or that cyclic AMP analogs act indirectly via transcriptional activation or phosphorylation of the transcription factor(s) involved in the C2 complex (Fig. 9). Since no CREs (cyclic AMP response element 5′-TGACGTCA-3′) appear to be present in the C2 binding sequence, we favor the latter interpretation.

The Role of TAAT Core Recognition Sequence in LAMB1 Gene Expression—Our data clearly demonstrate that a portion of the LAMB1 enhancer contains a cluster of AT-rich boxes...
RA binds to RXR, resulting in transcriptional activation of a homeodomain gene (Fig. 9).

Further Analysis of the "Late-acting RA-inducible Enhancer"—The role that this "late RA-associated enhancer" plays in the regulation and control of the tissue-specific expression of the LAMB1 gene remains to be investigated. Cloning of the gene(s) encoding the enhancer element-binding protein(s), especially the protein involved in the formation of the C2 complex, will allow us to define more precisely the molecular mechanisms involved in the regulation of the LAMB1 gene. In addition, the tissue-specific expression of the LAMB1 gene will be investigated by studying the expression of LAMB1 lacZ fusion genes in transgenic animals. This will allow us to determine what role this late RA-inducible enhancer plays in regulating the level of LAMB1 gene expression in various cell types.
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