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A mouse renin promoter containing the conserved decanucleotide element binds the same B-cell factors as an authentic immunoglobulin heavy chain promoter

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A mouse renin-1 gene promoter fragment, normally inactive in B-cells, becomes a potent promoter in these cells after insertion of the highly conserved decanucleotide (dc/cd sequence) of immunoglobulin heavy and light chain promoters [(1987) EMBO J. 6, 1685–1690]. We observe retarded complexes of the same electrophoretic mobility when the cd-containing renin promoter fragment or an authentic immunoglobulin heavy chain promoter fragment is incubated with a nuclear extract from myeloma cells, suggesting that the renin promoter is activated due to its acquired ability to bind a B-cell-specific positive factor. No retarded complexes are observed with the original renin promoter fragment thus questioning the presence of a repressor as an explanation for its lack of activity in B-cells.

Transcription factor; Promoter region; Immunoglobulin; Gene expression regulation

1. INTRODUCTION

The highly conserved sequence ATGCAAATNA (cd) is present upstream of the immunoglobulin heavy chain genes and, in the opposite orientation (dc), in the corresponding region of the immunoglobulin light chain genes and in the immunoglobulin heavy chain enhancer element [1,2]. Binding of nuclear factors to the above regulatory elements has been reported [3–6]. Some of these factors are specific to cells of the B lineage while others are ubiquitous [5,6].

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Abbreviations: dc/cd, decanucleotide element; Ig, immunoglobulin; poly(dI-dC), poly(deoxyinosine-deoxycytosine); Ren, renin; V_H promoter, immunoglobulin heavy chain promoter

As shown in our laboratory, insertion of the cd sequence into the 5′-flanking region of a gene not normally transcribed in myeloma cell lines, in occurrence the mouse renin gene, was sufficient to confer a strong transcriptional activity to this promoter in B-cells but not in non-lymphoid cells [7]. Here, we compare the binding of nuclear factors to the cd-containing renin promoter (Ren-cd), the intact renin promoter (Ren) and an authentic immunoglobulin heavy chain promoter (V_H promoter). Using an electrophoretic mobility shift assay we show that the engineered renin promoter and the V_H promoter behave in an identical manner in the presence of a B-cell nuclear extract or of an extract of non-lymphoid origin. However, no binding is observed with the intact renin promoter.

2. MATERIALS AND METHODS

2.1. *DNA probes*

Isolation of a V_H promoter fragment (V1) and of

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the renin promoter fragment (−142 to +30 with respect to the main transcription startpoint) has been described [7,8]. Insertion of the decanucleotide (cd sequence) into the renin promoter fragment is also described in [7]. The fragments were end-labelled with [α-^{32}P]deoxynucleotides using the large fragment of *E. coli* DNA polymerase.

2.2. *Cell culture and nuclear extract preparation*

Myeloma cells X63Ag8 secreting Ig were grown in suspension in Dulbecco's modified Eagle medium containing 10% fetal calf serum. Nuclear extracts were prepared according to Dignam et al. [9]. The HeLa cell nuclear extract was a generous gift from Dr C. Kedinger.

2.3. *Electrophoretic mobility shift assay*

The ^{32}P-labelled fragments (0.1–0.5 ng, 10000 cpm) were incubated for 30 min at room temperature with 8 μg nuclear extract protein in a reaction mixture that contained 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 7.5 μg poly(dI-dC) and 5% glycerol. At the end of the incubation period, glycerol was added to a final concentration of 10% and the samples were immediately loaded onto a 4.5% polyacrylamide gel (acrylamide/bisacrylamide, 39:1, w/w) containing 0.1 M Tris (pH 8.3), 0.1 M boric acid and 2 mM EDTA. The gel had been pre-electrophoresed for at least 30 min at 11 V·cm^{-1}. The samples were electrophoresed for 10 min at 15 V·cm^{-1} and then for 2 h at 11 V·cm^{-1}. The gels were fixed, dried and autoradiographed at 70°C with an intensifying screen.

3. RESULTS AND DISCUSSION

X63Ag8 is a myeloma cell line that secretes IgG 1 [10]. Nuclear extracts prepared from these cells were incubated with the different radiolabelled fragments followed by polyacrylamide gel electrophoresis. Bands of reduced mobility were obtained with the immunoglobulin heavy chain promoter fragment (V1) and with the cd-containing renin promoter fragment (Ren-cd) but not with the original renin promoter (fig.1). The V1 and Ren-cd fragments displayed an identical pattern of bands, a pattern that resembles that described in other laboratories for the binding of

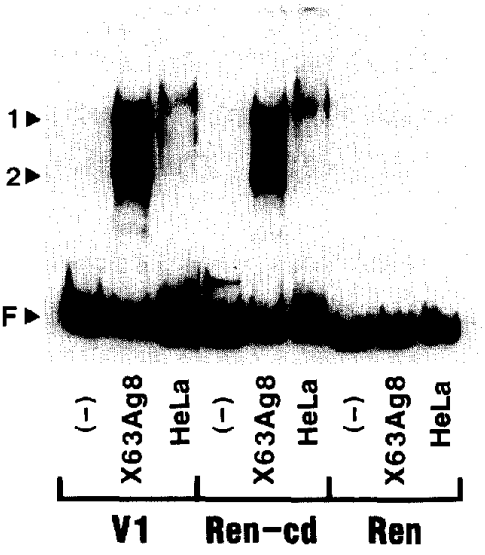

Fig.1. Electrophoretic mobility shift assay comparing the binding of factors to the V1, Ren-cd and Ren fragments. (−) No extract; X63Ag8, myeloma cell extract. Assay conditions are described in section 2.

nuclear extracts of lymphoid origin with immunoglobulin heavy or $\varkappa$ light chain promoter fragments [5,6]. Two major bands are observed (nos 1,2 in fig.1) as well as other minor bands. Incubation of the V1 or Ren-cd fragments with an extract from HeLa cells gave only one major band with a mobility similar to or slightly lower than that of band 1 observed with the X63Ag8 extract. Again no specific retardation of the original renin promoter fragment was observed. It is therefore possible that band 2 observed with the X63Ag8 extract corresponds to the B-cell-specific complex described in [6] whereas band 1 corresponds to a complex caused by an ubiquitous protein [5,6].

The bands of retarded mobility obtained with the V1 and Ren-cd fragments could be competed away by addition of excess unlabelled fragment to the incubation mixture (not shown). Moreover, the bands obtained with the V1 fragment were competed away by addition of excess unlabelled Ren-cd fragment, those obtained with the Ren-cd fragment being competed by the V1 fragment (fig.2). A synthetic oligonucleotide (20-mer) containing the cd sequence was an efficient competitor of the V1 and Ren-cd fragments and formed complexes of retarded electrophoretic mobility when incubated with the X63Ag8 extract.

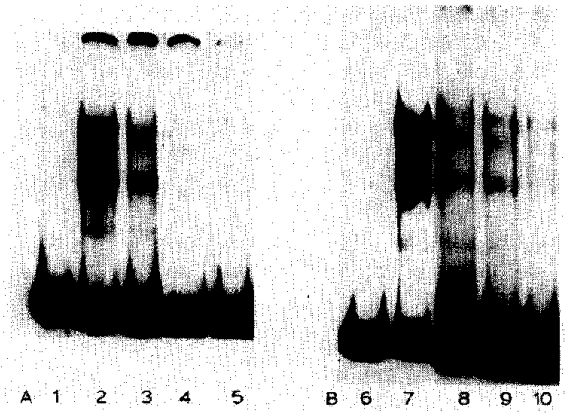

Fig.2. Binding competition analysis. (A) The V1 fragment (0.5 ng) was incubated with the X63Ag8 extract as before in the absence or presence of unlabelled Ren-cd fragment. Lanes: (1) no extract; (2–5) 0, 1, 2.5, 5 ng Ren-cd, respectively. (B) The Ren-cd fragment (0.5 ng) was incubated with the X63Ag8 extract in the absence or presence of V1. Lanes: (6) no extract; (7–10) 0, 1, 2.5, 5 ng V1, respectively.

The above observations strongly suggest that the presence of the decanucleotide sequence is sufficient to produce all the retarded complexes in the electrophoretic mobility shift assay. This is concordant with the recent observation by Landolfi and co-workers [11] that the retarded complexes obtained by incubation of lymphoid cell extract with a V_H promoter fragment are all due to binding of the factors with the nucleotides of the dc/cd sequence. Moreover, computer comparison of the sequence of V1 and Ren-cd fragments shows that the only region of strong homology between the two is the cd sequence (not shown).

The fragment of the renin promoter that was used in this work shows little promoter activity in lymphoid cells even when coupled to a functional enhancer. However, after insertion of the cd sequence, this fragment becomes a potent promoter in B-cells provided it is associated with a functional enhancer [7]. We can suggest from the experiments presented here that the engineered renin fragment becomes an active promoter in B-cells because the insertion of the cd sequence has given it the capacity to bind to a factor that is essential for promoter activity in these cells. This factor could be the one that produces complex 2 of fig.1, i.e. the B-specific factor. An alternative interpretation would be that insertion of the cd sequence activates the renin promoter by disruption of a repressor-binding site. However, such binding could not be shown with the original renin promoter fragment with either the myeloma cell extract or the HeLa extract (fig.1) even when the amounts of non-specific competitor DNA (poly(dI-dC)) used in the assay were reduced (not shown).

Although the cd sequence has been shown to be important for the activity of several promoters in non-B-cells [12–16], introduction of the cd sequence did not confer a strong promoter activity to the renin fragment when assayed in a mouse fibroblastic cell line [7]. Formation of the retarded complex produced by the ubiquitous factor that binds the cd sequence (band 1 in fig.1 and see [5,6,11]) may not be sufficient for a strong promoter activity in fibroblasts. It is therefore possible that in the cases where binding of the ubiquitous factor to the cd sequence is important for promoter activity in non-B-cells, other sequences and/or factors might be implicated as well.

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