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The duck β-globin gene cluster contains a single enhancer element

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An erythroid-specific enhancer was previously identified in the 3′-flanking region of the β adult gene in chicken and duck, by transfection into AEV transformed chicken erythroblasts. Here we show that the duck enhancer is equally active in erythroid human K562 cells, presenting an embryonic/fetal program of globin gene expression. Furthermore, no other enhancer was found within the 20 kb of DNA including four β-like globin genes as well as a 1.5 kb upstream and a 3 kb downstream sequence.

Globin gene; Enhancer; (Duck)

1. INTRODUCTION

The cluster of β-globin genes in the genome of chicken and duck contains four genes which are expressed in a tissue-specific and developmental stage-dependent manner [1]. In both species this cluster includes from the 5′- to the 3′-side: the ϱ-gene (embryonic), the β-H gene (hatching), the β-A gene (adult) and the ϵ-gene (embryonic) [2,3].

DNA sequences controlling the specific expression of globin genes have been detected upstream and downstream from the genes [4,5]. Enhancers are among the 'cis' elements responsible for tissue-specific gene expression [6]. An erythroid-specific enhancer was identified in the 3′-flanking region of the β-A gene in chicken and duck [7–9]. The localization and the nucleotide sequence of this enhancer are conserved in the two species; in a 180 bp segment the sequence homology exceeds 80% [9]. The enhancer is located between the adult (β-A) and embryonic gene (ϵ) placed further downstream. This fact raised the possibility that the enhancer might be involved in the differential expression of these genes during development. Previous work with the chicken enhancer has indicated a preferential activity in red cells expressing the adult globin genes [7].

In an attempt to extend these studies, we decided to test the activity of the duck enhancer in embryonic cells and chose the human erythroid line K562 as a model system [10]. Furthermore, we were also interested in determining whether additional enhancer elements are present within the duck β-globin gene domain. The four genes are quite similar and cross-hybridize with each other. In view of the differential control of the embryonic and adult genes one might ask whether analogous control elements exist in the proximity of the other globin genes.

Here we present evidence that the duck enhancer is active in the human erythroid line K562. Therefore the trans-acting factors involved in its action are present in embryonic-type cells and are conserved between the human and avian erythroid cells. Furthermore we found that within 20 kb of DNA, the duck β-globin domain contains a single enhancer element.

2. MATERIALS AND METHODS

All recombinant plasmids were constructed by standard methods [11]. The different fragments were inserted into the vector pA10CAT2 [12] at the *Bgl*II site, which was filled in by the Klenow fragment of DNA polymerase I; the pA10CAT2

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vector contains the CAT (chloramphenicol acetyl transferase) bacterial gene and the SV40 early promotor.

Chicken erythroblasts of the line LSCCHD4 transformed by the wild-type AEV clone 6C2 [13] were grown at 37°C in Dulbecco's modified Eagle's medium (Gibco) supplemented with 8% fetal calf serum and 2% chicken serum. Human K562 cells [10] were grown in RPMI 1640 medium supplemented with 10% fetal calf serum.

Cell transfections were performed by the DEAE-dextran method including a DMSO treatment [14].

For the CAT assay [15], cell extracts were prepared by three cycles of freezing and thawing. Samples corresponding to 40 μg of total protein were incubated for 3 h. The reaction was terminated by an ethyl acetate extraction. Finally, the acetylated forms were separated from unmodified [^{14}C]chloramphenicol by thin-layer chromatography on silica gel plates (chloroform/methanol, 95:5). Chromatograms were autoradiographed, and the identified spots were cut out and counted for radioactivity in a scintillation counter. The percentage of acetylated chloramphenicol versus total counts was calculated.

3. RESULTS AND DISCUSSION

The duck β-A globin enhancer was initially identified in a 2.2 kb *Bam*HI fragment starting within the second intron of the β-A (adult) globin gene and extending 1.3 kb downstream from the poly(A) site (fig.1). Subsequently, the DNA segment having the enhancer activity was narrowed down to a 500 bp *Ava*I-*Cla*I fragment, located 70 bp downstream from the polyadenylation site ([9] and fig.1). In order to better define the borders of the enhancer, the original 500 bp fragment was cut by the enzyme *Dde*I, and the resulting subfragments were cloned in pA10CAT2 and assayed for enhancer activity in the AEV transformed chicken erythroblasts, as described before [9]. The enhancer activity was found in the central 350 bp long fragment which appears to be much more active than the initial 2.2 kb fragment (20-fold versus 5-fold stimulation of CAT activity, fig.2A). This 350 bp fragment contains the 180 bp segment of maximum sequence homology between the duck and chicken enhancers.

3.1. *Enhancer activity in K562 cells*

K562 are human leukemic cells with an embryonic/fetal program of globin gene expression [16]. In these cells, upon induction by hemin, the amount of embryonic and fetal, but not adult hemoglobins, is increased, seemingly due to transcriptional activation [17,18]. We used the K562 cells to test the activity of the duck globin enhancer in an embryonic environment.

The *Dde*I 350 bp fragment which contains the duck β-globin enhancer was introduced into the K562 cells by transfection using DEAE-dextran [14]. Fig.2B shows that the duck enhancer is active in the human embryonic-like K562 cells, and it appears to be even more active than in the transformed chicken cells when compared to the SV40 enhancer. As shown in the tables of fig. 2A and B, the 350 bp fragment containing the duck globin enhancer stimulated CAT expression 40-fold in K562 cells whereas it showed a 20-fold stimulation in AEV transformed chicken cells. The SV40 enhancer and promoter, in contrast, seem to be substantially weaker in K562 cells. This stronger expression might be due to the fact that the K562 cells are more advanced in the erythroid pathway than the AEV-transformed chicken erythroblasts. Indeed, K562 cells contain low amounts of fetal and embryonic hemoglobins even before induction by hemin, while the AEV transformed erythroblasts used do not [13–16].

The activity of the duck enhancer in the K562 cells suggests that the factors which are involved in this effect are conserved between humans and birds. These factors are most probably DNA binding proteins [19,20] which recognize the nucleotide sequence of the enhancer; one might hence assume that they are common to adult and embryonic cells. Alternatively, the enhancer might contain separate control modules required for adult or embryonic activity. Interestingly, several attachment sites for nuclear DNA-binding proteins have been found in the chicken enhancer, most of which are lying within the DNA segments conserved between chicken and duck. They bind differentially factors from embryonic or adult cells [21].

3.2. *The duck β-globin gene cluster contains a single enhancer*

It was of importance to know whether multiple enhancers exist in the β-globin genes cluster, an arrangement which might influence differentially the expression of embryonic and/or adult genes. Therefore, several restriction fragments, encompassing the entire gene cluster as well as 1 kb upstream and 3 kb downstream, were cloned in the pA10CAT2 vector. These recombinants were then used to transfect AEV transformed chicken erythroblasts and K562 cells. None of the

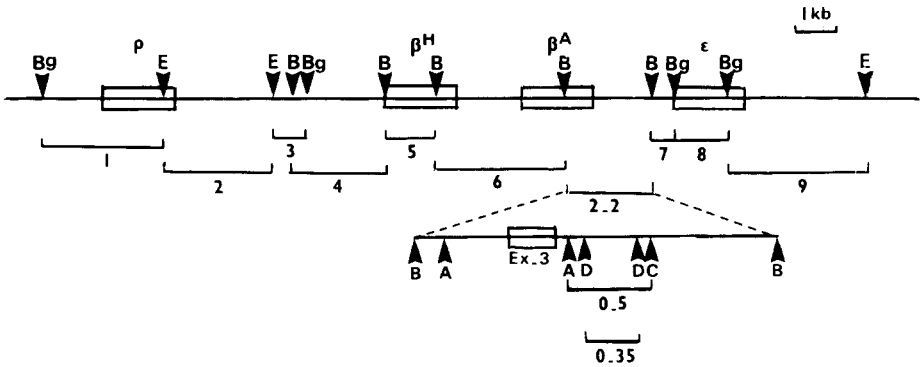

Fig.1. Genomic map of the duck β-globin gene cluster [3] indicating fragments 1, 2, 3, 4, 5, 6, 7, 8, 9 and also the 2.2 segment and its sub-fragments 0.5 and 0.35 which were tested for enhancer activity. Restriction sites are: A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; D, *Dde*I; E, *Eco*RI. Ex 3, the third exon of β-A globin gene.

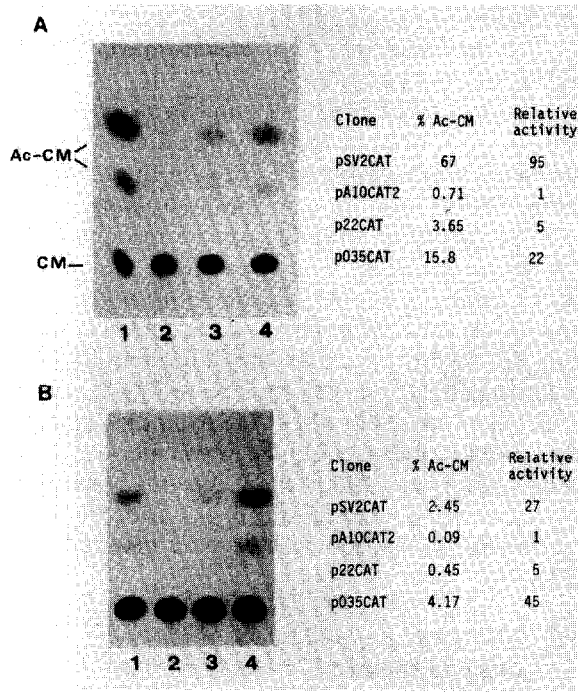

Fig.2. CAT activity found in (A) AEV transformed chicken erythroblasts, and (B) in K562 human erythroid cells after transfection by recombinant: (1) pSV2CAT which contains the SV40 enhancer; (2) pA10CAT2, the enhancerless vector; (3) p2.2 CAT containing the duck β-globin enhancer in a 2.2 kb fragment; and (4) p0.35 CAT, containing the 0.35 kb fragment of (3). The result of thin-layer chromatograms on silica gel plates are shown. The percentage of CM conversion and the relative activity of the different samples are given in the two tables.

fragments showed increased CAT activity in comparison with the enhancerless vector after transfection, either in the chicken erythroblasts (not shown) or in the K562 cells (fig.3).

Therefore, the enhancer located on the 3′-side of the β-A gene seems unique in the β-globin gene cluster. It cannot be ruled out that some enhancer element escaped detection by being cut or put near negative sequences, or that other control elements might exist further upstream or downstream. Nevertheless the former possibility seems unlikely since dividing the enhancer did not abolish its activity (not shown).

The fact that the β-A globin gene enhancer described here is the only one present (or so far identified) in the duck β-globin gene cluster, and the fact that it is active in adult and embryonic-type erythroid cells, makes it tempting to speculate that this enhancer might be involved in the activation of more than one gene. Enhancers have often been shown to act on two different genes [22]. In the human β-globin gene cluster three enhancer elements were discovered: one located in the 3′-flanking region of the fetal γ-A globin gene [23] and two others in the 3′-part of the adult β-globin gene [24]. The latter elements are considered to be developmentally specific. In the case of the chicken β-globin enhancer, it has been clearly shown that it is necessary for the expression of the adult β-A gene [8]. The possibility of action of this enhancer upon the embryonic ϵ gene situated on its 3′-side was, however, not excluded (see also [21]). In the

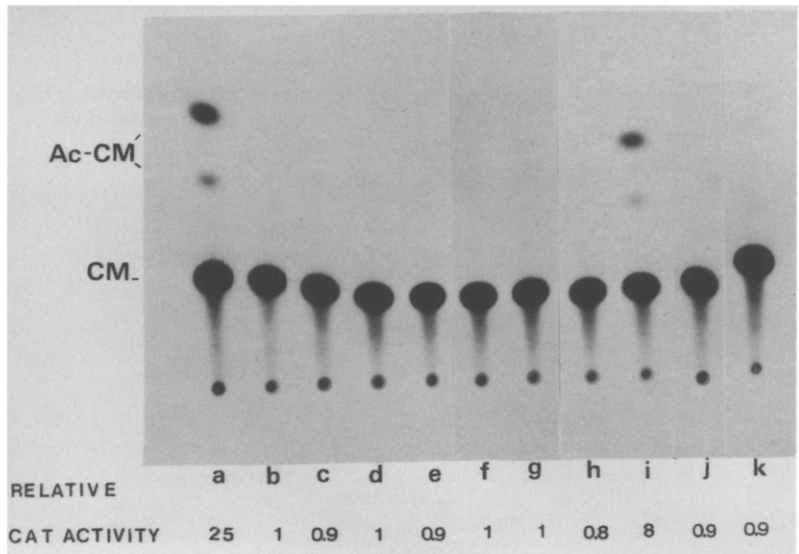

Fig.3. CAT activity in K562 cells after transfection with recombinants containing the fragments shown in fig.1. (a) pSV2CAT, (b) pA10CAT2, (c) p1CAT, (d) p2CAT, (e) p3CAT, (f) p4CAT, (g) p5CAT, (h) p6CAT, (i) p22CAT/1, (j) p8CAT, (k) p9CAAT. Fragment 7 was previously found negative [9].

case of the duck globin enhancer we have not yet addressed the question of action on a particular gene. However, the absence of additional enhancer elements in the entire duck β-globin gene cluster favors a simple model for avian globin gene clusters in which a single enhancer element operates on different genes.

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