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# Enhancer and promoter activity in the *JH* to *IGHM* intron of the Pekin duck, *Anas platyrhynchos*

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**Abstract**

A transcriptional enhancer, Eμ, was defined in the *IGH* locus of the Pekin duck, *Anas platyrhynchos*. Regions of DNA from the *JH* to *IGHM* intron were cloned into reporter constructs containing the SV40 promoter and transiently transfected into chicken B and T lymphocytes. A strong transcriptional activity, of several hundred-fold greater than that of a reporter construct with the promoter alone, was localized to a 281 bp region that contains 2 E-box motifs, CAGCTG. This fragment showed enhancer activity in both orientations and was active in chicken B cells but not in T cells. When the activity of the enhancer was tested in constructs without a promoter, it showed high transcriptional activity in the forward orientation, but much less activity (by two orders of magnitude) when tested in the reverse orientation. This suggests that the fragment contains not only enhancer activity but may contain promoter activity analogous to that of the Iμ promoter described in mammals. Thus it appears that the location, but not the fine structure, of the Eμ enhancer was established before the evolutionary divergence of the avian and mammalian lineages some 300 Myr ago.


*Keywords:* Aves; Ducks; Immunoglobulin genes; Transcriptional enhancer; I-exon promoter; Evolution

## 1. Introduction

The nature of transcriptional control in the vertebrate *IGH* locus is well understood for very few species, having been investigated primarily (and exhaustively) in the mouse and human [1–6]. In these two mammalian species it is clear that transcriptional control is intimately involved not only in the expression of transcripts encoding a functional heavy chain polypeptide, but also in the events of *V–D–J* recombination, class switching, and somatic hypermutation [7–12]. Little is known of the evolutionary history of transcriptional control in the *IGH* locus, and detailed investigations of enhancers in this locus have been conducted in relatively few non-mammalian species of vertebrates, with most information being available for the teleost fish, particularly the catfish and zebrafish [13–17]. The teleost fish permit interesting comparisons to be made, as they represent the earliest-diverged vertebrate lineage that shares with mammals the "translocon" arrangement of their *IGH* locus [18,19]. Two major conclusions have emerged from these studies of teleost fish: first, that the *IGH* enhancers of fish, while functionally similar to those

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of mammals (i.e. they are B-cell specific) are located within the $\mu$ (*IGHM*) to $\delta$ (*IGHD*) intron, a position incompatible with class switching by chromosomal recombination as this would result in loss of the enhancer [13,15,16,20]. Second, the fine structure of fish *IGH* enhancers is very different from that of any of the mammalian *IGH* enhancers [13,16,17,21]. Although the core region of mammalian Eμ enhancers contains μA, μB, Oct, E-box and μE3 (or CBF) motifs [22], the teleost *IGH* enhancers (Eμ3′) function through a combination of octamer and μE5 sites [13,16,17,21]. Significant evolutionary divergence in the transcriptional control elements of a locus (as opposed to relatively strong conservation of coding elements) is commonly but not universally observed [23]. In the case of the *IGH* locus our understanding of the evolutionary history of its transcriptional control is incomplete, because information is missing for several major, critical groups of vertebrates. Two of these are the amphibia and the birds. Amphibia such as *Xenopus* spp. are the descendants of the earliest vertebrates to colonize land, but the amphibia are also the earliest-derived animals to possess an *IGH* locus of the translocon form that shows classical class–switch recombination [24]. In contrast, while the *IGH* locus of birds also has a translocon arrangement [25,26] it relies primarily on gene conversion, rather than *V–D–J* recombination, to generate the primary repertoire of antigen binding sites [27]. This distinctive method of generating diversity used by the birds has implications for the position of the transcriptional enhancer; because the region 5′ of the single functional *VH* gene is not subject to deletion during *V–D–J* recombination in birds, a functional enhancer could be located a short distance upstream of this *VH* gene, from which position it would be close enough not only to the *VH* gene, but also to the *C* region genes, enabling it to carry out its full range of functions, in *V–D–J* rearrangement, class switching and somatic hyper mutation, as defined in the mammals [9,10,12,28,29]. Thus, enhancer function could be present in any one of three positions in the avian *IGH* locus: upstream of the functional *VH* gene; in the *JH* to *IGHM* intron; and downstream of *IGHY*, the most 3′ of the C region genes [25,26].

Given our knowledge of the structure and function of the *IGH* locus of the mammals, the most straightforward hypothesis regarding enhancer function in birds is that it occurs in the *JH* to *IGHM* intron. This present study was undertaken to test this hypothesis for the *IGH* locus of the Pekin duck (*Anas platyrhynchos*).

## 2. Materials and methods

### 2.1. Cell lines

Chicken cell lines, DT40 (a B cell, [30,31]) and 132B (a T cell, [32]) were maintained in Iscove's Modified Dulbecco's Medium with GlutaMAX (Gibco, Grand Island, NY) and supplemented with 1% chicken serum (Sigma, St. Louis, MO), 5% fetal bovine serum (Hyclone, Logan, UT), and 50 U/50 μg per ml each of penicillin and streptomycin (Gibco, Grand Island, NY), respectively. All cell lines were maintained under the conditions recommended by the supplier (American Type Culture Collection, 〈http://www.atcc.org/〉), at 37 °C in a humidified 5% carbon dioxide atmosphere.

### 2.2. Transfections

The chicken B- (DT40) and T (132B) cell lines were transiently transfected by electroporation. $1–2 \times 10^6$ cells in 180 μl of serum-free medium were mixed with 20 μl of DNA in TE buffer. Electroporation conditions were 170 V, 1100 μF for the two chicken cell lines. Experimental constructs, positive control (pGL3-control, Promega, Madison, WI) and negative control (pGL3-promoter, Promega, Madison, WI) were all transfected in equimolar ratios, standardized to the equivalent of 8 μg per transfection for the positive control construct. As a transfection efficiency control, the *Renilla* luciferase-expressing pRL construct (Promega, Madison, WI) was co-transfected at 0.5 μg. The experiments also included a mock transfection (TE buffer, no DNA) to determine auto-luminescence. Transfected cells were cultured for 24 h at 37 °C before being harvested and assayed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI). The samples were read in a luminometer (20/20 instrument, Turner Designs, Sunnyvale, CA), and the light units were adjusted for auto-luminescence, normalized for transfection efficiency and calculated as fold activation compared to the negative control, or as % activity of the duck Eμ enhancer.

### 2.3. Enhancer region constructs

A putative enhancer containing region was identified in the duck *JH* to *IGHM* intron from