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Evolution of antibody class switching: Identification and transcriptional control of an Iυ exon in the Duck (*Anas platyrhynchos*)☆

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Abstract

Immunoglobulin class switching is characteristic to the tetrapod lineage, but the nature of this process has been elucidated only in mammals, where I-exon transcription initiates and directs the recombination in the IgH locus. Here, it is shown that an I-exon occurs 5′ of the υ (IgY constant region) gene of the duck (*Anas platyrhynchos*): it is longer than mammalian I-exons and comprised primarily of tandem repeats. The Iυ promoter was identified and shown to be responsive to stimulation with IL-4 but not LPS. It contains Oct, LYF-1, ATF, and C/EBP motifs. Site directed mutagenesis indicates that 2 C/EBP motifs are uniquely necessary for the response of the promoter to IL-4, as tested in the mouse pre-B cell line, 70Z/3. These results support the conclusion that the signal transduction pathways controlling I-exon promoter responses to cytokines have been highly conserved in vertebrate evolution.

Keywords: Class switch; Evolution; Avian; Interleukin 4

1. Introduction

In the vertebrate antibody response, diversity of ligand recognition is achieved through sequence variation in the variable (V) domains of the heavy (H) and light (L) chains. In addition, diversity of secondary biological function is achieved through the process of class switching, in which a B lymphocyte switches the isotype of expressed H chain. Whereas IgM and IgD are produced by alternative pathways of

Abbreviations H, heavy; L, light; V, variable; C, constant; bp, basepair; S, switch; VNTR, variable number tandem repeats; INR, initiation region.

☆ The nucleotide sequences have been submitted to the EMBL database with the accession numbers AJ517505, AJ534872 and AJ534873.

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processing of a primary transcript that includes both μ and δ sequences [1], switching from IgM to other antibody classes (in mammals IgG, IgA and IgE) is achieved by chromosomal recombination [2]. While the mechanism of class switching has been described only in mammals, the evolutionary history of this phenomenon is of great interest, particularly since it is now recognized that both class switch recombination and the generation of high affinity binding sites in the Ig-V regions are mechanistically linked [3]; they both require the expression of activation-induced cytidine deaminase [4]. In mammalian B-cells, the switch from expression of IgM to the production of other antibody classes (except, typically, for IgD) is dependent on the expression of germ-line transcripts of the constant (C) region gene targeted for switching. These germ-line transcripts lack V-encoding sequences, contain the C region exons, and originate with *I*-exons 5′ of the switch (S) regions [5]. An *I*-exon is located 5′ of the S region for each C region gene, is typically 100–500 bp in length and contains multiple stop codons in all three reading frames. The function of the *I*-exon is not fully understood as it can readily be exchanged with an unrelated sequence provided that splicing to the C region exons is maintained [6]. This has led to the conclusion that it is not the I-exons per se, but rather the sterile transcription and RNA processing, especially stabilized R-loop structures of the sterile transcripts [7], that are important for the class switch to occur. The germ-line transcripts are driven from the TATA-less promoters of the *I*-exons. While elements within the $E\mu$ enhancer have been shown to promote the constitutive production of μ germ-line transcripts [8], the *I*-exon promoters of the other heavy chain genes, (γ, α, and ε), are induced by cytokines following B-cell activation. In vitro treatment of mitogen activated mouse B-cells with combinations of cytokines has led to the conclusion that class switching can be directed to specific isotypes by specific sets of stimuli. For example, IL-4 induces switching to IgE and IgG1 [9–11], TGF-β promotes the production of IgA [12–14] and IgG2b [15]; while IFN-γ targets the IgG2a [16] and IgG3 genes [17]. There are recent reports suggesting that the target of the class switch machinery also involves, besides the cytokine-induced transcription factors, DNA binding factors that distinguish between the different S regions [18]. The C region genes are typically all in the same transcriptional orientation. Thus, class switch recombination involves a looping-out mechanism in which the intervening DNA is deleted as a circle [19]. Although class switch recombination is believed to occur not only in mammals but also in amphibia, reptiles and birds [20,21], the mechanisms by which this process is initiated, directed and controlled are not known for any non-mammalian species. The organization of the IgH locus in the Pekin duck [22,23], and chicken [24], presents interesting questions regarding the control and mechanism of class switching in birds. This is because the IgH locus is organized with the C genes in the order μ–α–υ, with the α gene in inverse transcriptional orientation. Thus, while the switch from μ (IgM production) to υ (IgY production) follows a looping-out/deletion mechanism, the switch to α (IgA production) must be dependent on the inversion of the chromosomal segment containing the μ and α genes [22,24]. In this study, the genetic structures that are predicted to underlie class switching to IgY in the duck (*Anas platyrhynchos*) have been addressed, to determine whether a cytokine-responsive I-exon homologue lies upstream of the υ gene.

2. Materials and methods

2.1. Animals and cell lines

American commercial ducks (MLF, Milford, IN) were the source of materials used in this study. Chicken cell lines, DT40 (a B-cell, [25,26]) and 132B (a T-cell, [27]), were maintained in Iscove's Modified Dulbecco's Medium with GlutaMAX (Gibco, Grand Island, NY) and supplemented to 1% with chicken serum (Sigma, St Louis, MO), to 5% with fetal bovine serum (Hyclone, Logan, UT), and with 50 U/50 μg per ml each of penicillin and streptomycin (Gibco, Grand Island, NY), respectively. The mouse pre-B cell line 70Z/3 [28] was generously provided by Dr Lucille London and grown in RPMI medium 1640 with GlutaMAX (Gibco, Grand Island, NY), supplemented to 10% with fetal bovine serum, 50 μM 2-mercaptoethanol and 100 U/100 μg per ml each of penicillin and streptomycin. All cell lines were maintained at 37 °C in a humidified 5% carbon dioxide atmosphere.