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Short Communication

Inspection of the 3′ UTR Genomic Region for RAG1 and RAG2 in Rainbow Trout (*Oncorhynchus mykiss*) Reveals Potential Regulatory Motifs

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In all vertebrates, antigen receptors are generated via site-specific recombination events of germline Ig and TCR V (D) J gene segments (Schatz et al., 1992). The recombination activating genes 1 and 2 (RAG1 and RAG2) are essential for this process, which generates the primary immune repertoire (Mombaerts et al., 1992; Shinkai et al., 1992). Both genes are expressed solely in primary lymphoid tissues and precursor lymphocytes. The genomic locus for the RAG genes has been conserved in both sequence and overall organization from teleosts through humans that shared a common ancestor some 450 million years ago (Hansen and Kaattari 1995,1996). The RAG genes are tightly linked and are transcribed in a convergent manner utilizing a common 3′ untranslated region (5′-RAG1 → 3′ UTR 3′ ← RAG2–5′). In trout, the lowest order for which the RAG genomic locus has been characterized, the common 3′ UTR is only 2.4 kb in length as compared to 5–11 kb in the higher vertebrates. In addition, the trout RAG genes utilize overlapping polyadenylation sites, which is unique in comparison to the higher-vertebrate RAG loci. To date, little is known about the factors or sequence motifs that govern the transcription of the RAG genes. Recently, Döbbeling and colleagues (1996) showed that a genomic fragment encompassing murine RAG1 and RAG2 contained regions responsible for their transcriptional regulation. Enhancer motifs by definition can be located 5′, 3′, or within a gene itself, so the trout RAG 3′ UTR was analyzed for sequence motifs that may regulate RAG transcription. This is the first report describing the entire RAG 3′ UTR genomic sequence from any vertebrate.

Two clones encompassing the trout RAG 3′ UTR were sequenced for this study, one derived during the isolation of RAG2 (Hansen and Kaattari, 1996) and the other from the amplification of trout genomic DNA by PCR using Elongase (BRL). The primers used in the PCR (R1R2 5′ CAGGAGGATGCTGACATG3′ and R2R1 5′ GAAGCGCTTCTTCAGGAG 3′) reactions are located near the stop codons within the trout RAG1 and RAG2 coding sequences. The amplified products (2.41 kb) were cloned into pCRII (Invitrogen). It was previously reported (Hansen and Kaattari, 1996) that the trout 3′ UTR for RAG1 and RAG2 was 2.8 kb in length, but in fact it is 2.39 kb. Both clones (Phage and PCR-generated) were sequenced using Sequenase V1.0 (USB) and analyzed using BLAST N/X 1.4.9MP in GCG (Altschul et al., 1990), SIGNAL SCAN V4.05

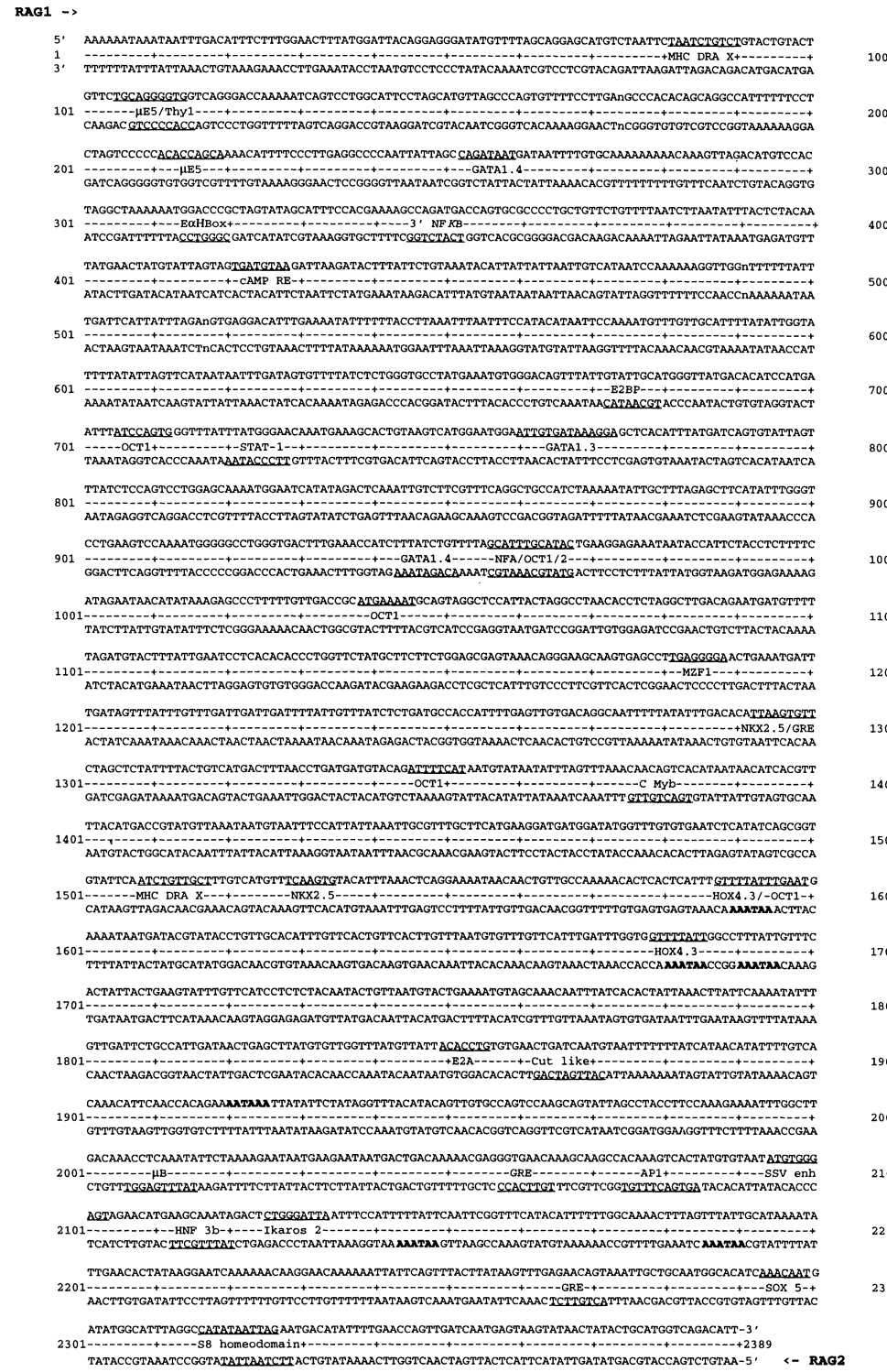

FIGURE 1 Sequence of the common 3′ untranslated region for the trout RAG locus depicting potential motifs (underlined on appropriate strand) involved in transcriptional regulation. The strand and orientation for RAG1 and RAG2 are depicted in bold with arrows. Polyadenylation sites are in bold (Hansen and Kaattari, 1996).

(Prestridge, 1991), and MatInspector V2.0/TRANSFAC 3.0 matrix (Quandt et al., 1995), both of which can be found on the WWW. As a control for motif-recognition specificity, the genomic sequence for trout RAG1 (3.9 kb) was used, which detected three conserved motifs, AP1fos/jun, 3′ NF*K*B enhancer, and the T-cell-specific Lyf-1 motif.

Both 3′ UTR clones were identical in size (2,389 bp) and only displayed 4-bp differences attributable to PCR errors. BLASTN/X searches did not reveal any major significant homologies. An inspection of the 3′ UTR (Fig. 1) using Signal Scan and MatInspector (matrix/core = 0.97) motif-recognition programs found an array of regulatory motifs (GATA, NFA, E2BP, Oct-1/2, NF-*K*B-en, AP1, STAT-1, Ikaros-2, NKX-2.5 (Tinman homologue), glucocorticoid response elements (GRE), HOX and cutlike homeodomain binding sites involved in lymphocyte regulation, development, and viral replication. Some of these elements (μE5, μB, and Octamers) were also found in the catfish IgH locus (Magor et al., 1994), which were shown to regulate tissue-specific expression in murine-transfected lymphocytes. Additionally, two variants of the MHC DRA X box were found, which are primarily utilized by MHC class II+ cells. Finally, it is indeed intriguing to note that both B- and T-cell-specific elements (Octamers and GATA1.3) were found within the trout RAG intergenic region, which may be related in some way to the lymphoid-specific expression of the RAG genes.

Whether the motifs described in this paper are essential or conserved in all vertebrates will have to await further analysis to determine their potential role in V (D) J recombination. Future efforts will include reporter constructs containing the trout RAG 3′ UTR to discern if it can regulate transcription in a tissue/lymphoid-specific manner.

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