Identification and expression pattern of a second isoform of the newt alpha retinoic acid receptor

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Retinoic acid is able to alter pattern in regenerating amphibian limbs by respecifying the positional memory of the regeneration blastema (1). Transcription factors of the nuclear receptor class that are responsive to all-*trans*-retinoic acid are favored to mediate these effects. Three subtypes of retinoic acid receptor (RAR) have been identified in vertebrates — RARγ and its urodele relative RARδ, RARβ and RARα, each occurring in multiple isoforms distinguished by their N-terminal sequences (1–3). This diversity suggests that a first task in the study of the mechanisms of positional respecification in regeneration is an inventory of RARs resident in the blastema and an assessment of their relative levels. The most abundant blastemal RAR is the δ receptor, which is present in multiple isoforms (4). RARα message is also readily detected in the blastema, but to date only one amphibian isoform, α_1, has been cloned (5). We report here the isolation of a RAR cDNA for a second α isoform from a newt tail library prepared in λZAP. Its complete nucleotide sequence is available under EMBL accession number Z14254. Sequence comparisons for the N-terminal region A (Figure 1) affiliate this RAR with the χ_2RAR paralogs, presenting 54% identity with the mouse α_2 receptor. This value is close to the 58% identity found between the newt δ_2 and mouse γ_2 receptors (4). The comparative values for the χ_1 receptors, by contrast, appear to range more widely; the newt α_1 region A is 76% identical with that of mouse α_1 whereas the newt δ1 receptor N-terminal is less than 45% identical with that of the mouse γ_1 receptor over their region of overlap (5).

The αRAR is broadly and fairly uniformly distributed across newt tissues (4). RNase protection experiments (Figure 2) demonstrate that the α_2 receptor is a major isoform in many newt tissues but not, apparently, in normal and regenerating forelimb. Interestingly, the α_2 receptor is a major contributor to αRAR message levels in long term cultures of hindlimb mesenchymal cells from thigh explants (TH4B cells, Figure 2) and proximal blastemas (B1H1 cells, not shown). This is the first clear difference in RAR expression patterns that we have identified between these culture models of blastemal mesenchymal cells and forelimb blastemas.

Figure 1. Amino acid sequence alignment for the N-terminal region A of the newt α_2 receptor, the mouse α_2 receptor (2), the newt δ2 receptor (4) and the mouse β2 receptor (3). Residues in common are shaded if present in the newt α_2 sequence and in bold type otherwise. Nv, *Notophthalmus viridescens*.

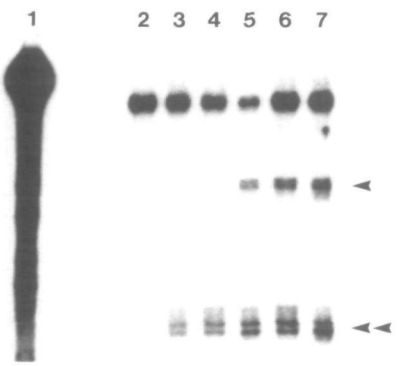

Figure 2. Expression of newt α_2 RAR studied with the RNase protection method. Lane **1**: pCBA2 antisense riboprobe. **2**: tRNA control; **3**: forelimb; **4**: distal forelimb blastema; **5**: kidney; **6**: liver; **7**: TH4B cells. 10 μg of total RNA per sample (see ref. 4 for methods). Riboprobe pCBA2 extends from the RARα C region *Pst*I site 36 bases into the α_2 A region. Bands signalled by single arrowhead indicate RARα_2 expression levels. Bands marked by double arrowheads identify RARα transcripts with A regions distinct from that of RARα_2. Experiments with similarly constructed α_1 riboprobes indicate that, particularly for lanes 5–7, most if not all of this signal is due to α_1 receptor message.

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