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

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Received August 21, 1992; Revised and Accepted October 6, 1992

EMBL accession no. Z14254

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 RARβ, RARα 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, α1, 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 XZAP. 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 X2RAR paralogs, presenting 54% identity with the mouse α2 receptor. This value is close to the 58% identity found between the newt δ2 and mouse γ2 receptors (4). The comparative values for the γ1 receptors, by contrast, appear to range more widely; the newt α1 region A is 76% identical with that of mouse γ1 whereas the newt δ1 receptor N-terminal is less than 45% identical with that of the mouse γ1 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 α2 receptor is a major isoform in many newt tissues but not, apparently, in normal and regenerating forelimb. Interestingly, the α2 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.

REFERENCES


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