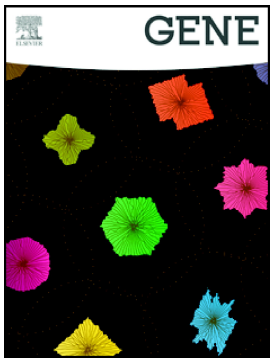

Molecular characterization and gene expression patterns of retinoid receptors, in normal and regenerating tissues of the sea cucumber, Holothuria glaberrima

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Title

Molecular characterization and gene expression patterns of retinoid receptors, in normal and regenerating tissues of the sea cucumber, *Holothuria glaberrima*

Abstract

Retinoic acid receptors (RAR) and retinoid X receptors (RXR) are ligand-mediated transcription factors that synchronize intricate signaling networks in metazoans. Dimer formation between these two nuclear receptors mediates the recruitment of co-regulatory complexes coordinating the progression of signaling cascades during developmental and regenerative events. In the present study we identified and characterized the receptors for retinoic acid in the sea cucumber *Holothuria* glaberrima; a model system capable of regenerative organogenesis during adulthood. Molecular characterizations revealed the presence of three isoforms of RAR and two of RXR as a consequence of alternative splicing events. Various analyses including: primary structure sequencing, phylogenetic analysis, protein domain prediction, and multiple sequence alignment further confirmed their identity. Semiquantitative reverse transcription PCR analysis of each receptor isoform herein identified showed that the retinoid receptors are expressed in all tissues sampled: the mesenteries, respiratory trees, muscles, gonads, and the digestive tract. During regenerative organogenesis two of the receptors (RAR-L and RXR-T) showed differential expression in the posterior segment while RAR-S is differentially expressed in the anterior segment of the intestine. This work presents the first description of the components relaying the signaling for retinoic acid within this model system.

Keywords: retinoic acid receptor, retinoid X receptor, retinoic acid, echinoderm

Abbreviations: aa, amino acid(s); bp, base pair(s); cDNA, DNA complementary to RNA; dpe, days post evisceration; DNase, deoxyribonuclease; dNTP, deoxyribonucleoside triphosphate; EST, expressed sequence tags; NR, nuclear receptor(s); ORF, open reading frame; RA, retinoic acid; RAR, retinoic acid receptor(s); RXR, retinoid X receptor(s); PCR, polymerase chain reaction; *UTR*, untranslated region(s); uAUG, upstream initiation codon(s); uORF, upstream open reading frame(s)

1. Introduction

The receptors for retinoic acid are members of the steroid and thyroid hormone superfamily of nuclear receptors (NR). These ligand-dependent transcription factors interact with DNA response elements controlling the enrollment of co-regulatory molecules that moderate transcriptional progression (Germain et al., 2006 a, b). The members of this superfamily activate various signaling cascades synchronizing developmental, metabolic, and physiological homeostasis in metazoans (Cotnoir-White et al., 2010). Signaling through the NR is mediated by steroid hormones (e.g. estrogen, glucocorticoids, vitamin D3), thyroid hormone (e.g. T3, T4), retinoids, or other small lipophilic molecules mainly derived