METAMORPHOSIS-ASSOCIATED ACTIVATION OF THE COLLAGENASE GENE IN ANURAN LARVAE.

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Collagens are the most abundant proteins of the extracellular matrix in vertebrates. Though the turnover rate of this protein is very low in normal tissues, its synthesis and degradation are accelerated in tissues that are in the process of remodeling such as regeneration (Dresden and Gross, 1970) and metamorphosis (Gross and Lapiere, 1962). Animal collagenase (EC.3.4.24.7, MMP I) first discovered in tail tissues of the bullfrog tadpoles (Gross and Lapiere, 1962) and purified from the animals (Nagai et al., 1966). Genes and cDNAs for collagenase were isolated from several mammalian species such as human (Goldberg et al., 1986, Collier et al., 1988), porcine (Krebs et al., 1990) and rabbit (Fini et al., 1987) but not from non-mammalian vertebrates.

During anuran metamorphosis, organs of the larval body transform into adult organs. This transformation is accompanied with the breakdown of the larval structure and is triggered by thyroid hormone (TH). Collagen remodeling is involved in this process, in which collagenase plays key roles. We have developed a series of studies to elucidate the molecular mechanism of the remodeling of collagen at the anuran larval metamorphosis.

The western or northern blot analyses showed that expression of collagenase increases in both the body and the tails skins during spontaneous or TH-induced metamorphosis. Immunohistochemical studies revealed that most of collagenase is synthesized in epidermal cells but some in also mesenchymal cells, although the level is much lower.

Nucleotide and the deduced amino acid sequences of bullfrog tadpole collagenase showed high similarity to the mammalian enzymes. Genomic clones of the enzyme were obtained and analyzed for their structures. The structure of this gene is unique in that the human MMP1 gene contains 9 introns, while the tadpole gene consists only 3 introns. This result suggests that the exons of collagenase gene separate into some pieces through the evolitional process. We have found AP1 and Sp1 consensus sequence in 5'-upstream region of the bullfrog collagenase gene; these sequences were also found in mammalian MMPs. In addition, T3-RE (TH-responsible element)-like sequences were found in the region. To our knowledge, the T3-RE has not been found in 5'-upstream-region of mammalian MMP genes.