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SYNTHESIS OF A NOVEL TYPE IX - LIKE COLLAGEN CDNA

Rutgers University The State U. of New Jersey (New Brunswick) PH.D. 1986

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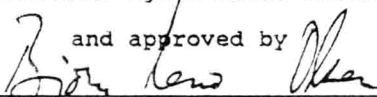
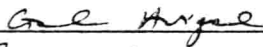
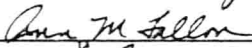
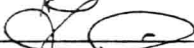
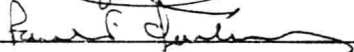
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SYNTHESIS OF A NOVEL
TYPE IX-LIKE COLLAGEN cDNA

by MARION KATHRYN GORDON

A dissertation submitted to the
Graduate School-New Brunswick
Rutgers, The State University of New Jersey
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Written under the direction of
Professor Bjorn Reino Olsen

and approved by

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ABSTRACT OF THE THESIS

Synthesis of a Novel type IX-like Collagen cDNA

by MARION K. GORDON, Ph.D.

Dissertation Director:

Professor Bjorn Reino Olsen

Type IX collagen is a heterotrimeric protein synthesized by chondrocytes, but not by fibroblasts. This disulfide bonded molecule is shorter than the fibrillar collagens and its collagenous triple helix is interspersed with non-collagenous domains.

The polypeptides synthesized by embryonic chick tendon fibroblasts include collagenous polypeptides in the molecular weight range of type IX collagen chains when analyzed by SDS PAGE. In order to elucidate the nature of these type IX-sized polypeptides, chick embryonic tendon fibroblast mRNA was used as a template to make a tendon short chain collagen cDNA.

Sucrose gradient fractions of 17 day old chick embryo tendon poly A⁺ RNA were translated in vitro. Fractions of RNA that were translated into collagenase-sensitive polypeptides smaller than pre-pro $\alpha 1(I)$ and pre-pro $\alpha 2(I)$ were used for cDNA synthesis. cDNAs were screened for the possible presence of gly-pro repetitive sequences using the restriction enzyme Sau 96I (GGNCC). Clones with

collagenous Sau 96I ladder patterns were mapped and labeled for Maxam-Gilbert sequencing, and also cloned into M13 for dideoxy chain termination sequencing.

The conceptual translation product of one clone, pMG377, showed remarkable homology to Type IX collagen chains. Two cysteinyl residues, close to the carboxyl ends of $\alpha 1(\text{IX})$, $\alpha 2(\text{IX})$ (from cDNAs), and $\alpha 3(\text{IX})$ (Michel van der Rest, personal communication) appear in analogous positions in pMG377. Two imperfections in the triple-helix of the Type IX chains appear in identical positions in relation to the cysteinyl residues in pMG377.

Clone pMG377 represents a partial copy of an mRNA specific for a novel type IX-like collagen mRNA synthesized by fibroblasts.

DEDICATION

In Memory Of

Samuel John Switz

John Hopkins Gainfort

William French Gordon

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