I. Development of fibrosis

CHAIRMAN: E. G. L. BYWATERS

Cell proliferation and collagen biosynthesis

D. S. JACKSON

From the Department of Medical Biochemistry, University of Manchester

The formation of fibrous tissue is the usual process of repair after most types of injury. In the simple situation such as the healing of a surgical scar the fibrosis restores the integrity of severed tissues and though the original tissue structure is not restored the end product is adequate both structurally and functionally. Nevertheless, there are circumstances in which there is an abnormal accumulation of fibrous tissue in the form of hypertrophic and keloid scar and peritendinous and intra-abdominal adhesions. In other situations such as liver cirrhosis and lung fibrosis the accumulation of fibrous tissue seriously interferes with the normal cell function of the affected tissue.

Cell proliferation

Fibrous tissue is the end product of the inflammatory response to injury, a response which leads to the invasion into the damaged area of proliferating fibroblasts which then lay down the structural components of fibrous tissue, the most important of which is collagen. The initial inflammatory response seems to be essential for the subsequent proliferation of fibroblasts. In the first few hours after wounding, for example, serum proteins, fibrin, and aggregates of platelets are present. Recent studies suggest that the presence of platelets results in the release of a factor which stimulates fibroblast proliferation. Neutrophils subsequently appear but are replaced by monocytes, which are the dominant cell type at 72 hours after wounding. The monocyte is the precursor of the macrophage. By inducing a monocytopenia by pretreatment with corticosteroids and antimacrophage serum monocytes can be prevented from entering the wound. The result is a prolonged inflammatory response, few fibroblasts are present, and collagen formation is delayed. In contrast, an induced neutropenia has no effect on the subsequent events in wound healing.

Thus the first two to three days sets the scene for the appearance and proliferation of the fibroblast, producing chemotactic factors which attract the

fibroblast precursor into the damaged area and stimulate the proliferation of these cells. The precursor of the fibroblasts, at least in wounds and granulomas, is not the monocyte, nor are adjacent fibrocytes involved. Instead an undifferentiated mesenchymal cell originating in the vascular tissue moves into the damaged area. As it does so it divides and develops the apparatus of a secretory cell—multiple nucleoli and extensive, rough endoplasmic reticulum and a large, randomly distributed Golgi apparatus. Cell mitosis continues until the cells come into contact, when mitosis and migration ceases and the synthesis of the components of fibrous tissue begins. In a wound this occurs about the fifth day.

Collagen structure

GENETIC POLYMORPHISM

All extracellular collagens so far investigated consist of three polypeptide chains (α -chains) which are wound around each other to form a special type of helix. Unusual features are the presence of hydroxyproline and hydroxylysine, both of which have important roles in defining the final fibre structure. A number of the hydroxylysine residues have sugars attached to them, these glycosyl residues being linked through the hydroxyl group. The overall picture of the collagen molecule (tropo-collagen) is of a long, thin, fairly rigid rod about 290×1.4 nm which can spontaneously aggregate into fibrils of the kind seen by electron microscopy in most connective tissues. These aggregates are stabilized by covalent cross-links, and it is these which give the fibrils their tensile strength. (see paper by A. J. Bailey, page 5).

In recent years it has become apparent that the cells of higher organisms contain several different structural genes directing collagen synthesis. Five different types of α -chains, which give rise to four different collagen types, have been identified in vertebrate tissues. There is some degree of specificity

Table I Collagen polymorphism in various tissues

Type	Molecular form	Tissue
I	$[\alpha 1(\text{I})]_2 \alpha 2$	Bone, dermis, tendon, cornea, dentin
II	$[\alpha 1(\text{II})]_3$	Cartilages
III	$[\alpha 1(\text{III})]_3$	Fetal and infant dermis, early scar tissue, granulomas. Cardiovascular system
IV	$[\alpha 1(\text{IV})]_3$	Basement membranes

NOTE Tissues mentioned contain predominantly collagen type indicated but many tissues contain more than one collagen type.

in the type of collagen found in various tissues (Table I), indicating the selectivity of gene expression for collagen biosynthesis in certain cell types.

In tissues such as bone, tendon, mature dermis, cornea, and sclera the chain composition is $[\alpha 1(\text{I})]_2 \alpha 2$ —that is, having two identical chains $\alpha 1(\text{I})$, the third member of the triple-helix having a different primary structure $\alpha 2$ and the complete molecule being referred to as type I collagen. The major collagen type in cartilage is type II, which has three identical α -chains of different primary structure to $\alpha 1(\text{I})$ and is designated $\alpha 1(\text{II})$, with the chain composition $[\alpha 1(\text{II})]_3$. Yet another type of interstitial collagen has been found, and is named type III. It too has three identical α -chains of unique primary structure, the molecule being designated $[\alpha 1(\text{III})]_3$. This collagen is also unusual in that it contains cysteine and is disulphide bonded. This type of collagen was first found in embryonic dermis. Postnatally there is a switch from type III

to type I synthesis, with the result that the bulk of the collagen in adult dermis is type I. Type III also occurs in the cardiovascular tissues, constituting, for example, some 50% of the collagen of the human aorta.

A class of connective tissues distinct from others is found in the basement membrane. From these a further type of collagen (type IV) has been isolated and characterized. This has three identical α -chains $\alpha 1(\text{IV})$ of unique primary structure and is designated $[\alpha 1(\text{IV})]_3$.

The collagen in fibrosis appears to contain both type I and type III collagen. Initially type III may be formed, with the subsequent addition of type I. This is similar to the process seen, for example, in the development of the dermis.

SECONDARY MODIFICATIONS

Another kind of variation is found in the proportion of lysine residues which are hydroxylated to hydroxylysine and the degree of glycosylation of these hydroxylysines. As can be seen in Table II, the variations may occur within a given collagen type as well as between the different types. Thus tendon collagen, which is type I, has 4–6 residues of hydroxylysine and only 2 carbohydrate molecules compared with corneal collagen, which is also type I, which has 10 residues of each. There are also differences to be found in the relative proportion of Hyl-Gal and Hyl-Gal-Glc. Initially formed scar and granuloma collagen has a higher hydroxylysine content and a greater degree of glycosylation than the usual type I and type III.

Table II Variations in contents of hydroxyproline, hydroxylysine, and hydroxylysine glycosides in several mammalian collagens

Mammalian collagens	Residues/1000				Percentage distribution of carbohydrate units	
	Hypro		Hyllys	Substituted hyllys	Hyl-Gal	Hyl-Gal-Glc
	4-Isomer	3-Isomer				
Type I						
Human sclera	88.6	—	6.4	1.7	30	70
„ dermis	92.1	—	3.6	1.4	30	70
„ tendon	85.9	—	8.7	1.4	40	60
„ cornea	82.9	—	8.7	6.4	30	70
Type II						
Chick cartilage	103	2	23	9.2	40	60
Type III						
Human aorta	125	—	5	—	—	—
Type IV						
Human glomerular basement membrane	130	11	45	36	5	95
Bovine anterior lens capsule	132	10	57	42	3	97

Biosynthesis of procollagen

SYNTHESIS OF PRIMARY STRUCTURE

As initially synthesized the polypeptide chains are significantly larger than the extracellular tropocollagen α -chains. The increase in size is due to the presence of amino- and carboxy-terminal peptides (which are non-helical) with molecular weights of 15 000 to 20 000 and 33 000 to 37 000 respectively. These precursor chains, named pro- α -chains, also differ from tropocollagen in containing cysteine.

HYDROXYLATION

The amino-acids hydroxyproline and hydroxylysine characteristic of all collagens are absent from the pro- α -chains initially synthesized. Specific proline and lysine residues are hydroxylated by prolyl and lysyl hydroxylases, both of which require Fe^{++} , α -ketoglutarate, O_2 , and ascorbic acid as cofactors. These enzymes are bound to the rough endoplasmic reticulum and the hydroxylation begins on nascent polypeptide chains attached to membrane-bound ribosomes.

GLYCOSYLATION

All vertebrate collagens contain glucose and galactose linked through the hydroxyl group of hydroxylysine residues, and hence glycosylation is subsequent to hydroxylation. The two hexoses are found as galactosyl hydroxylysine or glucosyl-galactosyl hydroxylysine. Galactosyl and glucosyl transferases are involved, galactose being first attached to hydroxylysine and glucose being attached to the galactosyl hydroxylysine to form a disaccharide. Both enzymes are membrane bound, largely to the rough endoplasmic reticulum but with a significant proportion of the enzymes bound to the smooth endoplasmic reticulum.

FORMATION OF INTER-CHAIN DISULPHIDE BONDS AND THE TRIPLE HELIX

Unlike extracellular type I and type II tropocollagen, the corresponding procollagens contain cysteine residues located in the N-terminal region. Procollagen is secreted by cultured cells in a triple helical conformation which contains interchain

disulphide bonds. Recent evidence indicates that soon after the pro- α -chains are released from the ribosomes into the cisternae of the endoplasmic reticulum interchain disulphide bonds are formed and the triple-helical conformation is rapidly assumed by the pro- α -chains. This assembly of pro- α -chains apparently begins in the cisternae of rough endoplasmic reticulum and is completed in the cisternae of smooth endoplasmic reticulum.

The different types of collagen seem to differ in the time required for synthesis and secretion, type I taking the shortest time and type IV the longest. The time of formation of the triple-helix follows the same pattern and, since hydroxylation and glycosylation can occur only while the pro- α -chains are in a random-coil configuration, these time differences may be important in deciding the degree of hydroxylation and glycosylation which takes place.

Secretion and fibril formation

Once the assembly of procollagen molecules is complete they are secreted via the smooth endoplasmic reticulum, including the Golgi apparatus, and thence into the extracellular space. There is some evidence that the procollagen is packaged into membrane-bound vesicles in which it is transported to the cell surface and released into the extracellular space. At this point the terminal peptides are cleaved by specific peptidases to produce extracellular tropocollagen. Before the loss of the terminal peptides the procollagen molecules are soluble under physiological conditions of temperature and pH, but after this loss the tropocollagen molecules are insoluble under these conditions and can interact spontaneously and precipitate. This process is analogous to the formation of insoluble fibrin from soluble fibrinogen. One further modification also takes place in the extracellular space—that is, the formation of the precursors of the stabilizing crosslinks. An oxidative deamination of specific lysyl or hydroxylysyl residues in the terminal regions occurs involving an amine-oxidase which requires Cu^{++} as a cofactor.

The subsequent reactions lead to the stabilization of the collagen by covalent cross-link. This final phase of collagen biosynthesis is dealt with in the next paper.