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Collagen Biosynthesis in Nonfibroblastic Cell Lines

(prolyl hydroxylase)

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ABSTRACT **Prolyl hydroxylase and other enzymes of collagen synthesis have been found in cloned cells, including epithelium, melanoma, liver, HeLa, pituitary, kidney, and neuroblastoma. The significance of collagen biosynthesis in nonfibroblastic cells lines is discussed.**

Synthesis of collagen differs from the synthesis of other proteins in many ways. One is that some amino acids are not incorporated directly. In this case the precursors, proline and lysine, are incorporated into the polypeptide chain and are then hydroxylated to hydroxyproline and hydroxylysine by specific hydroxylases. Its unusual biochemistry provides many markers for collagen synthesis. As expected, both collagen chain formation and prolyl hydroxylase have been demonstrated in cultured fibroblasts that are associated with formation of the components of connective tissue. Green and Goldberg (1) first noted that collagen is also synthesized by cultured cells of nonfibroblastic origin. These included the following cell types: HeLa, KB (epithelial), F1 (amnion), HFH-18 (melanoma), and RPMI no. 3460 (melanoma). They demonstrated that these nonfibroblastic cells produce the collagen chain as well as prolyl hydroxylase. Their finding of considerable biosynthesis of collagen by nonfibroblastic cell lines is certainly unexpected. However, since many of their cells were not cloned, there remained the possibility of contamination by fibroblasts. The present study was undertaken to repeat the studies of Green and Goldberg and to extend them to other nonfibroblastic lines, using established cloned cell lines only. In addition, cellular prolyl hydroxylase was measured as a marker and compared to the formation of hydroxyproline by the cells.

Cells were obtained from the sources shown in Table 1 and were grown under conditions described previously (2). Some lines were assayed directly after they were thawed out of liquid nitrogen, without culturing. Stored cells had been scraped at highest growth density before they were frozen. Hydroxyproline was determined in the entire culture (cells plus medium) after hydrolysis, by the colorimetric method of Prockop and Udenfriend (3). Incorporation of [^{14}C]proline and transformation to [^{14}C]hydroxyproline was also measured as a much more sensitive method of detecting collagen chain formation. Collagen formation is expressed as the amount of [^{14}C]hydroxyproline produced, as measured by the method of Peterkofsky and Prockop (4). Prolyl hydroxylase activity was measured by the tritium release method, with a tritium-labeled chick-embryo substrate (5). The specificity of prolyl hydroxylase activity in each cell line was demonstrated by showing its dependence on 2-oxoglutarate. The latter is an absolute and specific requirement of the enzyme.

Table 1 shows that the specific marker for collagen biosynthesis, prolyl hydroxylase, appeared in all the cloned nonfibroblastic cell lines investigated, even in such highly differentiated cells as neuroblasts. Activity in all cells was high, and in neuroblasts it was almost as high as in the fibroblasts (1). In addition, formation of collagen hydroxyproline paralleled the activity of prolyl hydroxylase (Table 2), indicating a rather direct correlation between hydroxylase activity and hydroxyproline formation. To the results presented above one may add the recent findings of Ross and Glomset (6), which show that smooth muscle cells derived from blood vessels are able to form collagen *in vivo*. Other components of connective tissue are also apparently synthesized by nonfibroblastic cells. Several authors have shown that both *in vitro* and *in vivo*, epithelial cells of the skin (7–10), smooth muscle cells (11), and epithelial cells of the gut (12) are able to form glycosaminoglycans.

The reason for the presence of prolyl hydroxylase and other enzymes of collagen synthesis in nonfibroblastic lines is not apparent. The cells used here, those reported to produce other components of connective tissue, and all cells of ectodermal or endodermal origin may have a capacity to produce collagen which is normally repressed. The activity in tissue culture may be an indication of derepression that is unique to cell culture. On the other hand it may indicate that all these cells normally make collagen and that smooth muscle cells are not the only nonfibroblastic cells that are capable of forming collagen *in vivo*. Perhaps fibroblasts are not as central to

TABLE 1. *Prolyl hydroxylase activity in various cloned cells*

Cell line	Prolyl hydroxylase activity (cpm per 4 × 10^6 cells)
Human skin epithelium CCL-19*	2,500
Melanoma mouse S91*	3,500
Leydig CCL-83*	4,300
Chang-liver CCL-3*	7,700
HeLa S3†	10,500
Pituitary tumor CCL-89*	15,000
Syrian hamster kidney CCL-10*	40,000
Neuroblastoma CCL-131*	54,000
Mouse fibroblasts L-929†	92,000

* American Type Culture Collection.
† Microbiological Associates.

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TABLE 2. *Comparison of cellular prolyl hydroxylase activity and hydroxyproline formation*

Cell line	Prolyl hydroxylase activity (cpm per 4 × 10^6 cells)	[^{14}C]Hydroxyproline formed (cpm per 4 × 10^6 cells)
Pituitary tumor (CCL-89)	15,000	5,800
Syrian hamster kidney (CCL-10)	40,000	8,800
Neuroblastoma (CCL-131)	54,000	9,200
Mouse fibroblasts (L-929)	92,000	15,100

the process of collagen formation as we have been led to believe. The concept that fibroblast invasion precedes massive connective tissue formation, as in fibrosis, cirrhosis, or wound repair, may have to be reevaluated. The apparent invasion of fibroblasts may be no more than local cells in which collagen synthesis has been increased by factors released by the injury.

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