volume (Pfeffer et al., 1980), an increased heterogeneity of cell sizes (Pfeffer et al., 1982), an increased frequency of lobed nuclei (Pfeffer et al., 1982), and an increased actin microfilament organization (Pfeffer et al., 1980; Bourgeade et al., 1981).

The molecular mechanisms governing interferon's antiproliferative activity is currently a matter of intense investigation. Samid et al., (1984) reported that the antiproliferative activity was correlated to a substantial decrease in c-Ha-ras gene expression in c-Ha- ras -transformed NIH3T3 cells. Later, Jonak and Knight (1984) reported that IFN treatment markedly inhibited c-myc expression in Daudi cells. Subsequent studies with other transformed cell lines (Einat et al., 1985a; Knight et al., 1985; Dani et al., 1985) and normal cells (Einat et al., 1985b) clearly show that IFN treatment potently inhibits the expression of c-myc, c-fos, ornithine decarboxylase, and c-Ha-ras. Proto-oncogene action has been clearly shown to be involved in the regulation of cell growth. Therefore, the finding that IFN treatment inhibits the expression of these genes may explain interferon's antiproliferative activity. The interferons are also potent modulators of extracellular matrix metabolism. For instance, IFN-γ induces the expression of urokinase-type plasminogen activator (Collart et al., 1986).

A startling discovery was reported by Zullo et al., (1985) in which they reported another interesting relationship between IFN and PDGF. They reported that not only are the competence genes (genes that are induced early in the progression from G₀ to G₁) regulated by IFN and dsRNA, but that PDGF stimulates the expression of a β-interferon-like gene. While the proto-oncogenes myc and fos are induced within one hour of PDGF treatment, the IFN accumulates slowly over 12 to 24 hours. This is consistent with earlier reports that IFN is induced in cells treated with other growth factors such as interleukin 1 and 2, colony-stimulating factor-1, epidermal growth factor, and fibroblast growth factor (VanDamme et al., 1985; Torres et al., 1982; Moore et al., 1984; Johnson & Torres, 1985). These growth factors, like PDGF, also rapidly stimulate the expression of the competence genes in cultured cells.
In a similar fashion, Zullo et al., (1985) report that PDGF treatment stimulates the expression of (2'-5')-oligoadenylate synthetase mRNA. The activity of the enzyme has also been reported to be increased in fibroblasts stimulated with epidermal growth factor or serum (Wells & Mallucci, 1985) and Lin et al., (1983) reported that anti-interferon antibody had a mitogenic effect.

These data suggest that proto-oncogenes and interferon function in a feedback loop to regulate cell growth in response to growth factors. While genes responsible for driving the cells into competence are expressed early in response to growth factors, (e.g., c-myc and c-fos), the induction of IFN and (2'-5') oligoadenylate synthetase is much slower and may thereby regulate the growth response, eventually, in the absence of further growth signals, resulting in the termination of proliferation.

If the molecular mechanisms regulating senescence and interferon action share common pathways, we might predict senescence and IFN action to be additive. Jasney et al., (1984) report that IFN treatment decreased the growth rate, increased the average cell volume, and decreased the uptake of TCA-soluble $^3$TdR to a similar extent in young and senescent fibroblasts. However, IFN treatment significantly decreased the DNA synthesis in the senescent cells at concentrations where no inhibition could be observed in young cells. This suggests that the biochemical pathways for growth inhibition in IFN-treated and senescent cells may overlap. At what level this overlap occurs remains to be determined.

Feltham et al., (1987) asked whether interferon's antiproliferative activity was being mediated by another separate protein. They isolated a protein of 12,000 daltons that they named fibroblast-derived growth inhibitor (FDGI) from the medium of dsRNA-induced fibroblasts. Apparently, synthesis and secretion of this protein are increased in IFN-treated cells. This protein, when added back to cultured human diploid fibroblasts, Namalva, HeLa, A549, or mouse L cells gave inhibition of DNA synthesis at less than 10 ng/ml. This level of inhibition approaches that reported for IFN and TGF-β.
An additional growth inhibitor has been isolated from the medium of density-inhibited 3T3 cells. This protein named "fibroblast growth regulator" (FGR-s for the soluble form) has a Mr of 13,000 and shows a 50% inhibition of cell proliferation at doses of 3 ng/ml using 3T3 cells as the target (Steck et al., 1982; Voss et al., 1982). Monoclonal antibodies have been prepared against FGR-s and it is reported that one such antibody is mitogenic when added to cultures of 3T3 cells, even in the absence of any exogenously-added FGR-s (Hsu et al., 1984). This would indicate that the addition of the monoclonal antibody neutralized the effect of endogenous FGR-s and reversed the density-dependent inhibition of DNA synthesis. We may therefore conclude that FGR-s acts as an autocrine antiproliferative protein involved in the density-dependent inhibition of cellular proliferation in 3T3 cells.

Harel et al., (1978, 1985) have been investigating the proteins secreted by dense cultures of 3T3 cells that may be acting as inhibitory mediators of density dependent inhibition. One such protein has been designated "inhibitory diffusible factor - 45 kDa" (IDF45). Studies of the method of action of IDF45 have now began to focus on proto-oncogene function. Blat et al., (1987) report that density-inhibited chick embryo fibroblasts (CEF) could be stimulated to synthesize DNA upon the addition of serum and that this stimulation could be inhibited by 94% by the addition of purified IDF45. Next, they infected the CEF cells with a mutant of RSV temperature-sensitive for transformation. They report that the stimulation of DNA synthesis induced by transferring the cells to permissive temperature could not be inhibited by IDF45. Therefore, it appears that serum stimulation of DNA synthesis can be inhibited by IDF45 whereas p60\(^{v\text{-src}}\) - mediated stimulation cannot. This is not surprising considering the fact that p60\(^{v\text{-src}}\) is a mutated form of the cellular homolog and perhaps not subject to the normal regulatory mechanisms. As mentioned below under uncharacterized growth inhibitors, the growth inhibitor from 3T3 cell plasma membranes is also relatively ineffective in inhibiting at least one kind of transformed cell (Wittenberger and Glaser, 1977).
Transforming growth factor-\(\beta\) (TGF-\(\beta\)) was originally isolated as a protein capable of causing phenotypic transformation of rat fibroblasts (Roberts et al., 1981; Tucker et al., 1983). Since then, it has been shown that TGF-\(\beta\) is produced in several different cell types including: the immune system (Kehrl et al., 1986 and Rook et al., 1986), connective tissue (Sporn et al., 1983; Ignotz and Massague, 1986; Roberts et al., 1986), and epithelial tissue (Masui et al., 1986; Shipley et al., 1986). Furthermore, it has been shown that essentially all cells possess the corresponding high-affinity receptor (Frolik et al., 1984; Tucker et al., 1984; Massague and Like, 1985).

TGF-\(\beta\) has a reported relative molecular weight of 25,000 daltons and is composed of two disulfide-bonded polypeptide chains of 12,500 daltons each. The receptor is a disulfide-bonded complex of 560-620 kDa molecular weight. Despite similarity of nomenclature, TGF-\(\beta\) has no sequence homology with TGF-\(\alpha\) which is an analog to epidermal growth factor.

TGF-\(\beta\) is frequently referred to as a bifunctional modulator of cell proliferation (Assoian, 1985; Roberts et al., 1985). It appears to have both stimulatory and inhibitory effects depending upon the cell target and the conditions of cell culture. For example, TGF-\(\beta\) promotes the anchorage independent growth of normal mouse AKR-2B and rat NRK fibroblasts, yet it inhibits their proliferation in monolayer culture (Moses et al., 1985; Roberts et al., 1985). Apparently, TGF-\(\beta\) is identical to a protein inhibitor isolated from BSC-1 African green monkey kidney epithelial cells termed BSC-1 growth inhibitor (Holley et al., 1980, 1983).

Numerous investigators have implicated the effects of TGF-\(\beta\) on extracellular matrix protein production as explanatory for its function. In particular, the expression of FN, collagen, and proteoglycan are markedly enhanced in both fibroblastic and epithelial cells after TGF-\(\beta\) treatment (Ignotz & Massague, 1986; Roberts et al., 1986). Ignotz and Massague (1986) have demonstrated that the anchorage-independent growth of normal fibroblasts is mimicked
by the exogenous addition of fibronectin, and that such growth is blocked by the addition of the synthetic peptide Gly-Arg-Gly-Asp-Ser-Pro which specifically blocks the FN - FN receptor interaction. Ignotz and Massague (1987) reported that TGF-β increases the level of expression of the FN receptor in parallel with FN, which probably explains the increased incorporation of FN into the extracellular matrix.

Nilsen-Hamilton and Holley (1983) examined the effect of BSC-1 cell-derived growth inhibitor (now known to be identical to TGF-β) on secreted proteins. They reported that concurrent with DNA synthesis inhibition, BSC-1 cells respond with a 12-fold increase in the synthesis of a 48 kDa protein they called "Inhibitor-Inducible Protein" (IIP48). This 48 Kd protein has since been demonstrated to be PAI-1 by Nilsen-Hamilton, (personal communication), and PAI-1 has been shown to be induced in other cells by TGF-β treatment (Laiho et al., 1987; Lund et al., 1987; Saksela et al., 1987). In addition to stimulating the production of ECM protein and PAI-1, TGF-β decreases the synthesis of both u-PA and t-PA (Laiho et al., 1986). We can therefore summarize the effects of TGF-β treatment on cultured fibroblasts as increasing the synthesis of FN, collagen, and proteoglycans, and decreasing extracellular proteolysis by increasing the synthesis of PAI-1, and decreasing that of u-PA and t-PA.

g. Premature Aging Syndromes

There are several genetically-inherited diseases that have features resembling premature aging. These syndromes may therefore be studied as models for the study of aging. Five of these syndromes will be discussed here; namely: Progeria (Hutchinson-Gilford syndrome), Werner's syndrome, Ehlers Danlos syndrome, cutis laxa, and DeBarsy syndrome.

Progeria is a rare autosomal recessive disease which shares many features of normal aging (DeBusk, 1972). While patients usually appear normal at birth and up to one year of age, soon thereafter aging-like symptoms appear. These include: severe growth retardation, baldness,
loss of subcutaneous fat (especially apparent on scalp where superficial vasculature becomes easily visible), disproportionately small maxilla and mandible, skin wrinkling, and mottled skin pigmentation. Aseptic necrosis of the head of the femur and subsequent hip dislocation, and resorption of the clavicles and terminal phalanges are also common. More life-threatening symptoms include atherosclerosis and osteoarthritis, generally leading to morbidity and mortality at an average age of 12. Approximately 80% of the deaths are due to congestive heart disease or myocardial infarction. While the exact metabolic defect responsible for progeria remains to be identified, there is evidence that the defect involves an extracellular matrix-related enzyme. For example, Kresse et al., (1987) demonstrated that skin fibroblasts from an individual possessing progeroid characteristics secreted an abnormal glycosaminoglycan-free form of decorin into the culture medium.

Werner's syndrome (sometimes called adult progeria) is an inherited disorder with many symptoms similar to those of progeria with the exception that they begin later in life (Werner, 1904; Thannhauser, 1945; Epstein et al., 1966; Brown, 1983). Patients generally have a normal childhood with symptoms appearing in the early teenage years. Symptoms include premature graying of hair, wrinkled and sclerodermatous skin, atherosclerosis, connective tissue calcification, osteoporosis, and cataracts. Patients generally die in the fourth decade due to myocardial infarction.

These symptoms have led many researchers to regard Werner's syndrome as an autosomal recessive connective tissue disease (Fleischmajer & Nedwich, 1973). In support of this, Zebrower et al., (1986) and Brown et al., (1985) reported that a patient with Werner's syndrome had greatly elevated levels of hyaluronic acid in the urine [this was previously shown to be the case with progeria as well, (Tokunaga et al., 1978)]. Bauer et al., (1988) noted that connective tissues in particular showed marked alterations such as scleroderma which was pronounced in acral areas. Bauer et al., (1986) reported that these findings might be explained in part by the observation that Werner's syndrome skin fibroblasts quickly
became unresponsive to growth stimulation by PDGF, but nevertheless secreted relatively high levels of collagenase activity into the culture medium even when not stimulated to do so with growth factor. These experimental results and the previously-mentioned clinical data strongly suggest that these premature aging disorders may be due to a metabolic defect in connective tissue matrix proteins in particular, those regulating the catabolic side of connective tissue maintenance.

The Ehlers-Danlos syndromes (EDS) present themselves in a heterogeneous manner, both genetically and clinically. In general, they share certain clinical features, such as hyperelastic skin, hypermobile joints, arterial rupture, and premature periodontal disease. There are at least nine variants described. All of these disorders involve a pathological alteration of the connective tissue matrix. In the case of EDS IV also known as acrogeria (Pope et al., 1980 & 1983), the defect is in the lack of sufficient production of type III collagen (Pope et al., 1970, 1975, & 1977, Byers et al., 1979). The relevance of EDS IV to aging seems quite straightforward. A similar decrease in the production of type III collagen relative to type I collagen can be seen in normally aging skin (Epstein, 1974; Epstein and Munderloch, 1975; Ramshaw, 1986).

The next progeroid syndrome I will discuss is termed cutis laxa. This disorder is diagnosed on the basis of a patient presenting with loose sagging skin with reduced elasticity (Uitto, 1985). The resulting wrinkled skin gives patients a greatly progeroid appearance in the face. Biochemical and histological studies indicate that patients with cutis laxa show a fragmentation and deficit of elastic fibers in the skin, lung, and other tissues (Sayers et al., 1975; Anderson et al., 1985). In addition to sagging, wrinkled skin, this disorder is characterized by diverticula in various organs, congenital dislocation of the hips, and osteoporosis (Agha et al., 1978). Recently, reduced elastin gene expression was observed in cultured fibroblasts from patients with cutis laxa (Olsen et al., 1988). This suggests that at least in some cases the disorder involves deficiencies at the level of elastin gene expression.
DeBarsy syndrome is an autosomal recessive disorder with characteristics in common with cutis laxa. DeBarsy et al., (1968) originally reported on a case of a girl with a progeroid appearance, dwarfism, oligophrenia, and degeneration of the elastic fibers of the skin and cornea with cataracts. The skin of these patients is thin (with a loss of subcutaneous fat), with mottled pigmentation, inelastic, and transparent. Collagen metabolism is apparently normal (Pontz et al., 1986), and therefore the etiology is currently thought to involve elastin metabolism, though the exact deficit is not known.

All of the above-mentioned premature aging syndromes appear to be inherited defects in connective tissue maintenance (Spence & Herman, 1973). Since they mirror a wide spectrum of age-related disorders, they deserve a great deal of attention. This underscores the need for a general study of the senescent fibroblast for possible diminished capacity to maintain the matrix. Next I will review the current understanding of fibroblast function in terms of its synthesis of extracellular matrix structural components and proteolytic enzymes and clarify how such a study can be accomplished.

C. The Role of the Fibroblast in Connective Tissue Maintenance

1. Growth Factor Modulation of Fibroblast Activity

The extracellular matrix plays an important role in the maintenance of structure in tissue, but it is not merely inert scaffolding. As I discussed earlier, extracellular matrix proteins strongly affect cell morphology. The extracellular matrix and its affect on morphology and cell spreading, in turn, appear to influence cell growth (Gospodarowicz et al., 1978,1980; Alema et al., 1985; Sawada et al., 1986; Rutka et al., 1987). For instance, it has been observed that reduction of cell spreading by manipulation of the extracellular substrate correlates with growth inhibition (O'Neill et al., 1986). Alterations of cellular morphology by the extracellular matrix also influences the differentiated state. For instance, chondrocytes are observed to