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Perspectives in Cancer Research

Growth Factors and Cancer[1]

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Overview

GFs[3] may be defined as polypeptides that stimulate cell proliferation through binding to specific high-affinity cell membrane receptors. These GFs differ from the well-known polypeptide hormones such as insulin and adrenocorticotropic hormone not only in the response elicited but also in the mode of delivery from the secreting to the responding cell. GFs do not usually act in an endocrine manner; they presumably diffuse short-range through intercellular spaces and act locally. Plasma contains few growth factors; several of those present in serum are presumed to be derived from platelets and are released during the clotting process (1–4). The presence of growth factors in platelets is thought to facilitate delivery of growth factors to sites of injury where they may play a major role in wound healing.

Besides being found in platelets, GFs are present in a variety of tissues, both adult and embryonic, and are thought to be released by many, if not all, cells in culture (5). Membrane receptors for growth factors are also highly ubiquitous with most cells having receptors for more than one growth factor (6–8). Growth factors have differing cell type specificities; some factors such as those of the hematopoietic system (*e.g.*, interleukin 2 or CSF-1) stimulate only one or a few cell types while others such as somatomedin C and EGF stimulate a wide variety of cell types, both epithelial and mesenchymal (see below). It has been demonstrated that multiple growth factors are required for maximum stimulation of specific cell types (9, 10). The requirement of nontransformed cells for more than one growth factor for proliferation is also supported by studies on the growth of cells in defined serum-free media. Unless the cells are neoplastically transformed, more than one growth factor supplement is necessary for growth (11–13). Exposure of a cell to one growth factor can lower the threshold for mitogenicity of a second growth factor (14). Moreover, growth factors operate at different points of the cell cycle (9, 10). For instance, transient treatment of fibroblasts with PDGF will induce a stable state ("competence") whereby cells are made responsive to other circulating plasma-derived factors (15). The multiplicity of growth factors in various tissues, the varying cell type specificity of GFs, and the requirement for multiple GFs for stimulation of specific cell types presumably provide the fine tuning of relative proliferation rates necessary for coordinated growth of cells to form tissues during development and to maintain tissues in the adult state.

Much of the impetus for study of GFs has come through their presumed involvement in cancer. Evidence for this involvement dates to early work showing a decreased serum requirement for growth of neoplastically transformed cells (16–18). With the advent of serum-free culture techniques and the availability of purified growth factors, the altered serum requirement in transformed cells could be translated into a diminished or absent requirement for specific growth factors (11, 19). Loss of requirement for specific growth factors is a common finding in many types of cancer cells (19, 20) and could be mediated by (*a*) the activation of autologous GF synthesis ("autocrine" activation), (*b*) synthesis of an altered GF receptor, or (*c*) activation of a post-receptor pathway that bypasses the GF receptor requirement.

Some of the more convincing evidence linking growth factors and cancer has come from recent work linking oncogenes and growth factors. One proto-oncogene, c-*sis*, codes for the B chain of PDGF (21, 22). Another (c-*erb*B) codes for the EGF receptor (23). Similarly, the product of the c-*fms* oncogene appears very similar to the CSF-1 receptor (24). Moreover, there is evidence to suggest that several other oncogene products are similar to growth factor receptors in that both have transmembrane and tyrosine kinase domains (25). Recent data indicate that the p21 *ras* oncogene protein is involved in transduction of the growth factor signal and may be an obligatory intermediate in this pathway (26). Growth factors have been shown to increase transcription of certain proto-oncogenes (*myc* and *fos*) (27–30), the products of which may in turn regulate the transcription of other genes necessary for stimulation of cell proliferation. These data suggest that many, if not all, of the oncogene products may be involved in the growth factor-receptor-response pathway and indicate points at which alterations may occur leading to the development of neoplastic transformation.

Many growth-active polypeptides that fit the definition of growth factors have been described, and this review will concentrate on several well-defined examples. The cellular response to growth factor binding and possible mechanisms of growth factor involvement in the neoplastic process including the oncogene relationship will be addressed.

Specific Growth Factors

EGF. EGF was first described by Cohen (31) as a peptide which would stimulate precocious eyelid opening and tooth eruption in newborn mice and was purified on this basis; its ability to stimulate the growth of cultured cells was recognized later (32, 33). First purified from male mouse submaxillary glands (31) and later from human urine as urogastrone (34, 35), mature

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[3] The abbreviations used are: GF, growth factor; ALV, avian leukosis virus; CSF, colony-stimulating factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor or somatomedin; IL, interleukin; NGF, nerve growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; p21, 21-kDa protein; cDNA, complementary DNA; NRK, normal rat kidney; Con A, concanavalin A.

EGF is a 6-kDa single polypeptide chain of 53 amino acids displaying 3 internal disulfide bonds (36). EGF is synthesized from a precursor which may be as large as 128 kDa (37). Radiolabeled 46- and 130-kDa species have been detected in mouse male submaxillary gland and mouse kidney, respectively (38).

The 4.8-kilobase EGF mRNA from male mouse submaxillary gland has been cloned and sequenced (37, 39). The cDNA clones define an open reading frame sufficient to code for 1168 (37) or 1217 amino acids (39). In both cases, native EGF is encoded in residues 977–1029 of the deduced amino acid sequence. Originally thought to have a limited range of tissue expression, recent *in situ* hybridization analyses of sections of whole newborn mice (38) indicate that RNA complementary to cloned EGF probes may be present in a large variety of tissues, including a surprisingly high expression in the distal tubules of the kidney. The protein translated from this mRNA in kidney remains as a high-molecular-weight protein; little or no 6-kDa EGF is detectable in this tissue (38).

Radioimmune (40) and radioreceptor (41) assays have been developed for measuring EGF concentration in extracts; the latter assay detects TGFα as equivalent to native EGF. Not only do EGF and TGFα (see below) both recognize the same cellular receptor, they are apparently equally effective on a mole-for-mole basis in most systems. It may be the case that EGF is the adult form of the embryonic growth factor TGFα. EGF is mitogenic for a variety of cultured mesenchymal and epithelial cells; its mitogenic activity is strongly potentiated by insulin (42, 43). EGF also acts in synergism with PDGF on BALB/c-3T3 cells (44). Aspects of differentiation are also induced following EGF treatment in certain cell culture models and *in vivo* (45, 46).

No tumors are yet known which synthesize EGF. Consistent with the oncodevelopmental concept which proposes that tumors may ectopically reactivate embryonic genes, all tumors and tumor cells which synthesize an EGF-like species, in fact, synthesize a similar molecule called TGFα, to be described later. An EGF-like molecule may also play a role in the benign hyperplasia induced by vaccinia virus which encodes a 140-residue protein processed to 47 residues showing significant homology to both EGF and TGFα, including conservation of the three internal disulfide bonds (47).

The cellular receptor for EGF is the best understood GF receptor and has served as a paradigm for other GF receptors. Although present on a large variety of cells, the EGF receptor was first purified from A431 cells (48), a cell line derived from a human squamous carcinoma which has an increased number of EGF receptors (7). The receptor is an integral 170-kDa membrane protein exhibiting an extracellular binding domain that serves to bind the ligand (EGF or TGFα), a transmembrane region, and an intracellular domain facing the cytoplasm exhibiting the tyrosine kinase function and presumably binding sites for ATP phosphorylation substrates (48). In response to EGF, the receptor is capable of autophosphorylation on tyrosine residues (49). A second form of the receptor missing the transmembrane domain is found in a secreted form in the A431 cell line (50), although the significance of this molecule is not clear. The oncogene v-*erb*B codes for a product homologous to a portion of the EGF receptor in which the EGF-binding domain has been deleted. Evidence exists suggesting that this truncation of the EGF receptor may lead to constitutive activation without requirement for ligand binding (see below).

Platelet-derived Growth Factor. PDGF is a major mitogen in serum; moreover, it elicits a chemotactic response in fibroblasts and smooth muscle cells (51). PDGF is a potent mitogen, sufficient in some cells to induce both DNA synthesis and cell division even in the absence of other growth factors (52). It is thought that most transformed mesenchymal cells produce PDGF or a PDGF-like molecule (53, 54).

PDGF purified from outdated human platelets is a mixture of polypeptides with molecular weights in the 30–32-kDa range. The platelet-derived dimer is composed of a 14–18 kDa A chain disulfide bonded to a 16-kDa B chain (21, 55); the size heterogeneity probably reflects differential degradation of the A chain ends as well as differential addition of carbohydrate side chains. The B chain (or PDGF-2) is encoded in the c-*sis* proto-oncogene (21, 22, 54, 56, 57); its cellular transcript appears as a 4.2-kilobase mRNA in denaturing gels. Parts of the human c-*sis* locus have been cloned from genomic libraries (58, 59). There are 7 exons to the human c-*sis* locus of chromosome 22, encompassing at least 23 kilobases of DNA; no promoter has yet been found (59).

Although PDGF from platelets is apparently a heterodimer, transformed cells may actually secrete a B-B homodimer. Sequencing of a c-*sis* cDNA clone reveals an open reading frame sufficient to encode 241 amino acids or 27 kDa of protein (57, 59). A dimer of pro-B chains could thus include 54 kDa of peptide; carbohydrate addition would presumably add to the size of this pro-B chain dimer. PDGF may be synthesized as a high-molecular-weight precursor (54, 59–61) which is presumably disulfide bonded and processed to the 32-kDa secreted form observed in cultures of osteosarcoma cells (60–62) and glioma cells (63).

A radioreceptor assay for PDGF has been developed (64–66) which affords a specific and sensitive quantitation of PDGF in extracts and conditioned media. Scatchard analyses of ^{125}I-labeled PDGF binding allows analysis of receptor number per cell (400,000 receptors/cell for human fibroblasts) as well as the dissociation constant (K_d) for the factor-receptor complex (10–1000 pM). The concentration of PDGF exerting half-maximal stimulation of DNA synthesis varies widely, between 11 and 310 pM (0.4–10 ng/ml). This large variation may reflect the interaction of other growth factors with the cell which may lower the cell's threshold of response to PDGF.

PDGF was originally purified from blood platelets where it is stored as a component of the α granules (67). PDGF synthesis has been demonstrated in large vessel endothelial cells (68) and aortic smooth muscle cells of newborn but not adult rats (69). The 4.2-kilobase c-*sis* transcripts are present in the cytotrophoblastic shell of human placenta, and placental explants synthesize a PDGF-like molecule (70). Cell lines cultured from early placentas also express cell surface receptors for PDGF and respond to exogenous PDGF with an activation of the c-*myc* gene and DNA synthesis (70). Since the cells of the cytotrophoblastic shell are the most invasive and proliferative normal cells known, the expression of PDGF receptors in this tissue may help account for their "pseudomalignant" phenotype (71).

Receptors for PDGF are found on a variety of mesenchymal cells (65, 66) as well as human placental cytotrophoblasts (70). Other than the trophoblastic cells, receptors for PDGF are not found on most epithelial cells (66). Stimulation of cells with PDGF induces an autophosphorylation of a 185-kDa protein (72) which

turns out to be the PDGF receptor (73). An antibody to phosphotyrosine has been used in the purification of the receptor from BALB/c-3T3 cells; purified receptors inserted into liposome reconstitute the GF binding characteristics of the native receptor (74).

Transforming Growth Factor Type α. TGFs can be defined operationally by their ability to stimulate the anchorage-independent growth in soft agar of cells which are otherwise anchorage dependent (75). This definition has led to the isolation and characterization of two very divergent molecular entities: TGFβ, a 25-kDa disulfide-linked homodimer (described below); and TGFα, a 5.6-kDa species consisting of a single chain of 50 amino acids (76). TGFα was first described as sarcoma growth factor, now known to be composed of both TGFβ and a 5.6-kDa species (TGFα) that are secreted into the medium conditioned by the growth of murine sarcoma virus-transformed cell lines and that compete with ^{125}I-labeled EGF for binding to a common cell surface receptor (77, 78). As it turns out, purified TGFα alone in serum-containing medium only weakly stimulates soft agar colony formation (79). The apparent colony-stimulating ability of sarcoma growth factor was presumably due to the interaction of TGFα and TGFβ on NRK indicator cells (see TGFβ below).

The sequence of native rat cell-derived TGFα shows a significant homology to both human and mouse EGF (76, 80). Like EGF, TGFα is presumably synthesized from a precursor; the open reading frame of the cloned human TGFα gene is sufficient to encode a protein of 160 amino acids of which residues 40–89 encode native TGFα (81). Transcripts of 4.8 kilobases have been detected in the cell line 1072 F57, derived from a human renal cell carcinoma (81). Besides being found in a variety of virally transformed cells, TGFα has also been demonstrated in a variety of nonneoplastic tissues, including human placenta (82) and mouse and rat embryos (83, 84). However, TGFα has thus far not been demonstrated in nonneoplastic adult tissues and may represent the embryonic form of EGF that is inappropriately expressed in certain neoplastic cells.

Transforming Growth Factor Type β. TGFβ is very different from TGFα in molecular composition, biological response elicited, and membrane receptor binding. TGFβ is one of the most interesting growth-regulatory polypeptides because it has been demonstrated to both stimulate and inhibit cell proliferation with the response obtained depending largely on cell type (85–87).

TGFβ was first described as a factor stimulating the growth in soft agar of AKR-2B (88) and NRK cells (89) that did not compete with ^{125}I-labeled EGF for receptor binding. Although TGFβ was active in the soft agar assay on AKR-2B (clone 84A) cells alone, the soft agar response of NRK (clone 49F) cells to TGFβ required the presence of EGF or TGFα (89). It was not clear until later that the TGF activity in the NRK and AKR-2B assays was due to the same molecule, now called TGFβ (1, 90, 91). NRK cells seem to be unusual in their requirement for EGF in the soft agar assay, and thus the EGF requirement originally included in the definition of TGFβ (89) has since been removed (92).

TGFβ has been purified to homogeneity from four sources including bovine kidney (93), human placenta (94), human platelets (95), and feline sarcoma virus-transformed rat cells (96). These sources reveal a 25-kDa disulfide-linked apparently homodimeric molecule. Derynck *et al.* (97) have cloned the gene for TGFβ from a human genomic library and from cDNA libraries derived from human term placenta and the human fibrosarcoma line HT1080. Amino acid sequencing of reduced platelet-derived TGFβ confirms the conclusion that the 2 chains of TGFβ are identical; in conjunction with the sequencing of overlapping cDNA clones, these studies define the native molecule as a homodimer of 2 disulfide-linked chains of 112 amino acids each (97). These studies furthermore suggest a precursor encoded in the 391-residue open reading frame defined by the overlapping clones where native TGFβ is encoded by residues 280–391.

The gene for TGFβ is transcribed into a 2.5-kilobase mRNA present in a wide variety of normal and transformed cells; its abundance in human peripheral blood lymphocytes is increased severalfold by mitogen stimulation (97). In addition, TGFβ protein itself has been detected in normal liver, lung, kidney, submaxillary gland, brain, and heart tissue as well as embryos and placenta (1, 89, 94, 98, 99). A number of cells in culture both produce TGFβ and have specific TGFβ membrane receptors, yet they do not constitutively exhibit the phenotype induced by adding TGFβ. A partial explanation for these observations has been provided by recent work demonstrating that the TGFβ released by cells in culture was in an inactive form; activation occurred irreversibly with acid treatment (100, 101). Some evidence has been presented that the inactive TGFβ precursor might have a higher molecular weight than the active molecule (92), perhaps through association with a binding protein in a manner analogous to that of somatomedin C in plasma (see below). Considering the ubiquity of TGFβ (and its receptor), activation of a precursor molecule could represent an important regulatory step in TGFβ action.

TGFβ is mitogenic for a variety of fibroblastic cell types in monolayer culture (52, 86, 87, 96). In AKR-2B cells, this mitogenic activity is apparently conveyed through an indirect action involving PDGF.[4] TGFβ will induce DNA synthesis in AKR-2B cells after a prolonged prereplicative phase of 24 h instead of the 12–14 h seen with PDGF or EGF (52). In this instance, the mitogenic action of TGFβ is proposed to be indirect, acting to induce c-*sis* expression (increasing rapidly at 4 h, although already apparent at 20 min after TGFβ addition) and the appearance of a PDGF-like activity within the medium (detectable first at 8 h); it is thought this induced PDGF is the direct mitogen, accounting for the delay in DNA synthesis of 12 h.[4] This interesting twist in the growth factor story not only suggests a mode of action for TGFβ involving PDGF but also provides support again for a model in which several growth factors might act in concert to increase the proliferative capacity of a cell.

If the mitogenic activity of TGFβ is mediated through PDGF, then it would not be expected that epithelial cells which do not have receptors for PDGF would be stimulated by TGFβ. Intriguingly, the action of TGFβ can be inhibitory to cell growth in certain circumstances. Evidence has been presented (85) indicating that TGFβ is the same molecular entity as the growth inhibitor described by Holley *et al.* (102, 103) in the medium conditioned by the growth of BSC-1 monkey kidney cells. The growth-inhibitory action of TGFβ has since been demonstrated for a variety of neoplastically transformed epithelial cells (86, 87). In certain circumstances, transformation of epithelial cells may involve a loss of the inhibitory response to TGFβ. Whereas the growth of normal human prokeratinocytes is inhibited by TGFβ in a serum-

[4] E. B. Leof, J. A. Proper, A. S. Goustin, G. D. Shipley, P. E. DiCorleto, and H. L. Moses. Induction of c-*sis* mRNA and platelet derived growth-like activity by transforming growth factor, type-β: a proposed model for indirect mitogenesis involving autocrine activity. Proc. Natl. Acad. Sci. USA, in press, 1986.

free medium (87), it has been shown that a squamous carcinoma cell line grown in the same medium is not inhibited by TGFβ.[5] This is consistent with a model in which the repression of a growth-inhibitory response in transformation might have the same consequences as the induction of a growth-stimulatory response (52, 87, 104).

Radioreceptor assays for TGFβ have recently been developed (105–107), allowing for the quantitation of dissociation constant (25–140 pM) and receptor number per cell (10,000–40,000). The TGFβ receptor was detected on a wide variety of cell types, both epithelial and mesenchymal (105). It is apparently quite different from either the EGF or PDGF receptors; affinity labeling of the receptor in mouse cells identifies a 565-kDa complex, which dissociates in the presence of disulfide reagents to two 280–290-kDa subunits (108). The receptor is apparently a glycoprotein (108) and shows slightly larger species in human cells (615 and 330 kDa, respectively, for unreduced and reduced receptor).

Other Transforming Growth Factors. In addition to TGFα and TGFβ, other TGFs have been described that appear to be distinct from TGFα and β. An acid-labile factor, TGFγ2, that stimulates the growth in soft agar of BALB/c-3T3 cells has been described (109). This factor has been purified and an amino acid composition has been determined (110). Another factor is the epithelial tissue-derived factor which stimulates the growth in soft agar of the carcinoma cell line, SW 13 (111). Interestingly, this factor is released into media conditioned by the growth of these same cells, suggesting the possibility that this TGF may be involved in autocrine growth regulation of this carcinoma cell line.

Insulin-like Growth Factors (IGF-I and IGF-II). First described as a "sulfation factor" by Salmon and Daughaday (112), somatomedin C is the best known member of a family of insulin-like peptides, ancestrally related to proinsulin (113); members include IGF-I and IGF-II. IGF-I corresponds to human somatomedin C and IGF-II corresponds to human somatomedin A and rat multiplication-stimulating activity. In the literature, however, somatomedin C still generally goes by the original name. Produced in response to circulating growth hormone, somatomedin C is one of the important growth factors found in serum and plasma (114) active in stimulating the proliferation of a large number of cultured cells (115). Supraphysiological concentrations of insulin (>100 nM) can replace the IGF requirement in defined media through cross-reaction with ubiquitous IGF receptors (116). Somatomedins apparently circulate in plasma noncovalently bound to a specific carrier protein (117). Somatomedins have been hypothesized to stimulate cell growth in an autocrine fashion (118). BRL-3A cells secrete large amounts of IGF-II into the medium (119); however, they do not require the IGF-II for proliferation and thus do not satisfy the autocrine hypothesis (120). Recent evidence argues for a paracrine or autocrine role for somatomedin C in the stimulation of fetal mouse growth (121). A monoclonal antibody to human somatomedin C has recently been shown to strongly inhibit the mitogenic effect of plasma on competent BALB/c-3T3 cells (122).

Somatomedin C (IGF-I) has been purified from human serum and sequenced (123); it is a single chain of 70 amino acids with 3 internal disulfide bonds. It has also been prepared in milligram quantities by solid-phase synthesis (124). The human genes for IGF-I and IGF-II have been cloned (125); a recombinant somatomedin C identical to human somatomedin C except for the substitution of methionine with isoleucine at position 59 is available commercially. Interestingly, the human IGF-II gene is, in fact, closely linked to the human insulin gene, on human chromosome 11, band p15 (126). The genes for both IGFs indicate that the native 7 kDa proteins may be processed from 15–21-kDa precursors; the 70-residue native IGF-I has a 130-residue precursor, whereas the 67-residue native IGF-II has a 180-residue precursor (125). It has been speculated that IGF-I may be an adult somatomedin, whereas IGF-II would be its embryonic counterpart (127). IGF-I and IGF-II each appear to have their own receptor to which they preferentially bind, although cross-reaction is seen at high GF concentrations (128). Chemical cross-linking of radiolabeled IGF to cells has allowed the definition of quite distinct molecular entities (128). The cellular receptors for IGF-I (type I receptors) show homology to the insulin receptor, a heterotetrameric 450-kDa complex consisting of two transmembrane β subunits (98 kDa each), each disulfide bonded to one α subunit (130 kDa each) (129). The α subunits provide the insulin- (or IGF)-binding domains (130), whereas the β subunits possess ATPase and tyrosine kinase activities (131). At least for the insulin receptor now cloned, both subunits are encoded in a single polyprotein cleaved posttranslationally to yield the heterotetrameric receptor. A cDNA encoding the insulin receptor polyprotein (1370 amino acids) has now been cloned (132). The α region shows a surprising homology to the extracellular domain of the human EGF receptor. Not so surprising, however, is the homology of the β domain to members of the *src* family of tyrosine kinases; homology is highest, however, with the *ros* oncogene (132). These homologies strongly suggest that one or more of these oncogenes may encode growth factor receptors. The type II receptor (preferential for IGF-II) is simpler, exhibiting only a 250-kDa component which may be single chain (133). Type II IGF receptors may not undergo ligand-induced down regulation (134).

Interleukin 2. Upon treatment of human peripheral blood T-cells with the lectin Con A, soluble factors are released that stimulate the proliferation of activated T-cells (135, 136). One factor, first called T-cell growth factor or TCGF and later interleukin 2, was isolated which supported the long-term *in vitro* culture of clonal populations of normal cytotoxic T-lymphocytes (136). Using mRNA from the overproducer tumor cell line JURKAT, the gene for human IL-2 has been cloned as a cDNA; the sequence indicates a peptide of 153 amino acids (137) that is cleaved to form the mature 133-residue secreted sialoglycoprotein displaying one internal disulfide bond (138). The human gene for IL-2 spans about 8 kilobases and consists of 4 exons; it shows no significant rearrangements in the JURKAT tumor cell line (139). In addition, a cDNA-encoding mouse IL-2 has been cloned which exhibits 76% homology at the amino acid level to human IL-2 with an open reading frame sufficient to encode a protein of 169 residues (140). Treatment of the JURKAT cells with Con A induces an IL-2 transcript of 1.5 kilobases (137). Stimulation with the lectin phytohemagglutinin results in a 30-fold induction of IL-2 transcription in normal human lymphocytes (141). Interestingly, the immunosuppressive drug cyclosporin A deactivates the IL-2 gene in phytohemagglutinin-induced JURKAT cells (142), sug-

[5] G. D. Shipley, M. R. Pittelkow, J. J. Wille, Jr., R. E. Scott, and H. L. Moses. Reversible inhibition of normal human prokeratinocyte proliferation by type β transforming growth factor/growth inhibitor in serum-free medium. Cancer Res., *46:* in press, 1986.

gesting a role for the activation of the IL-2 gene during T-cell activation.

Cell surface receptors for IL-2 have been purified from both normal and transformed lymphocytes (143); although the receptor molecules are slightly different in size (55 and 60 kDa, respectively), the significance of this difference is unclear. This receptor is apparently quite different from those described for other growth factors; the sequencing of cloned cDNAs (144, 145) indicates an open reading frame of only 272 amino acids (33 kDa), containing a cytoplasmic region of only 13 residues, insufficient to encode a tyrosine kinase activity (144). The cytoplasmic domain does, however, contain one serine and one threonine which can be phosphorylated. The discrepancy between the observed size of purified receptor (55 kDa) and this open reading frame capable only of coding for 33 kDa of protein is problematic; 22 kDa of added carbohydrate would be surprising. However, it is possible that neither cDNA clone represents a functional IL-2 receptor; the functional receptor cDNA encoding a significant cytoplasmic domain may remain to be cloned. More puzzling yet is the observation that HuT-102B2 (human T-cell leukemia virus 1 transformed) cells contain an additional mRNA in which a 216-base region has been spliced out; this mRNA could encode a 200-residue protein identical to the normal IL-2 receptor except for a deletion of 72 amino acids at or near the presumed IL-2 binding domain (144). The significance of this alternative receptor species is not clear, although this might be similar to the truncated EGF receptor coded for by the *erb*B oncogene that is missing the ligand-binding site. In addition, the so-called anti-Tac antibody recognizes both a canonical, functional form and an alternative form of the receptor which displays 100-fold lower affinity for IL-2 (146). Both the 272-codon cDNA and the 200-codon cDNA have been transfected into COS cells; the larger cDNA transfectants both bind anti-Tac and radiolabeled IL-2, although with a 1000-fold lower affinity than expected (145). The 200-codon transfectants seem to produce neither functional receptor nor Tac-reactive material. Intriguingly, treatment of human lymphocytes with IL-2 induces a down regulation of canonical IL-2 receptors but at the same time induces an increase in the amount of the alternative receptor on the cell surface (146). In conclusion, the current status of the cloning and purification of IL-2 receptors has thus failed to provide a clear understanding of either receptor structure or its genetic regulation.

Functional receptors for IL-2 are not found on resting T-cells (147); the action of Con A or antigen in T-cell proliferation thus involves the induction not only of IL-2 production by the T-helper cells but also of IL-2 receptors on T-killer cells, a two-step process (148). The control of IL-2 receptor presentation in the immune response is in this way a key control of normal T-cell proliferation.

Fibroblast Growth Factors (Heparin-binding Growth Factors). Extracts of bovine neural tissue contain growth factors mitogenic for cultured fibroblasts and vascular endothelial cells (149). Reported by Gospodarowicz *et al*. (150) in bovine pituitary and then in bovine brain (151), the molecular characterization of these factors has been elusive until recently. It was claimed at one time that FGF was derived from brain myelin protein fragments (152); it has now been shown that this claim was mistaken (153). There are several factors present in these neural extracts which have been given the name FGF; they are all apparently single-chain proteins in the 14–18-kDa size. Other members of the FGF family include the factors described as endothelial cell growth factor, chondrosarcoma growth factor, and heparin-binding growth factors. Proteins that are apparently acidic and basic in the neural extracts show similar features (153–155). Several factors have now been purified to apparent homogeneity; an acidic form of FGF from bovine brain (156) and a cationic form from bovine pituitary (157) have been isolated by multistep procedures and an NH_2-terminal sequence was reported for the cationic form (158). Both factors have a molecular weight of 16,000.

Several factors that have properties similar to those of FGF have been recently purified by heparin affinity chromatography. An 18-kDa endothelial growth factor from chondrosarcomas was the first to be purified by this technique (159). Subsequently, it has been shown that the cationic 16-kDa pituitary FGF and cationic brain FGF can be purified by this technique and are identical (160). An 18-kDa form of the heparin-binding growth factor has also been observed in preparations from bovine pituitary (161) and hypothalamus (162). Others have reported that multiple forms of FGF activity can be isolated by heparin affinity, including both the cationic and anionic FGFs from brain (163). An amino acid composition for both forms has been reported (162) and the acidic form of the molecule isolated by this technique has the same molecular weight and amino acid composition as the molecule isolated by the multistep procedure (156). The complete sequence of bovine pituitary basic FGF is now available (164); the sequence describes a molecule of 146 amino acids (16.4 kDa). This sequence agrees with the partial sequence obtained for bovine basic FGF obtained from other tissues, including brain, adrenal gland, retina, corpus luteum, and kidney (164). Since this sequence differs substantially with that reported for the bovine acidic form (165), there are probably at least two genes encoding FGFs corresponding to the acidic and basic FGFs. However, there is antigenic and sequence relatedness between these two gene products (164). Furthermore, there is a slight amount of homology between acidic FGF and interleukin 1 (165). The factor described as endothelial cell growth factor is related to the FGF family. Purified endothelial cell GF has been radioiodinated for use in a receptor assay, allowing the estimation of dissociation constant (200–800 pM) and receptor number per cell (20,000–40,000) (166).

A radioreceptor assay for FGF might allow for a survey of the distribution of FGF content and FGF production by various tissues; no such survey has yet been done. The significance of an endothelial cell growth factor concentrated in brain or pituitary is yet unclear. The possible scenario of FGF as an endocrine growth factor would stand in contrast to patterns of other GFs as locally produced and locally acting paracrine or autocrine growth factors. The production of a FGF by chondrosarcoma is more in keeping with the general scheme, if one imagines a paracrine role for this growth factor in the stimulation of tumor angiogenesis, as has been suggested (167).

Nerve Growth Factors. Although NGF has been around as a defined substance for a number of years, its role as a factor for the maintenance and differentiation of sensory and sympathetic neurons argues against its inclusion in a strict list of growth factors. Nevertheless, NGF fits into the general scheme of growth factors in many ways. Indeed, recent evidence indicates that NGF may play a mitogenic role in cultured rat adrenal chromaffin

cells (168). First detected as a factor released by transplanted tumors (169), NGF was first purified from snake venom (170) and then mouse submaxillary gland (171). NGF isolated from submaxillary glands is found in a 7S complex, containing three protein subspecies labeled α, β, and γ (172). NGF activity resides in the β chain, a 26-kDa dimer of two identical NGF chains (118 amino acids per chain) which has been sequenced (173). Sequencing of mouse and human cDNA clones suggests that NGF is synthesized as a much larger precursor (174); proNGF is apparently a dimer of 307 residues per chain, with native NGF encoded in residues 188–305 of the precursor.

Receptors for NGF are present on a variety of normal sympathetic and sensory neurons as well as normal and neoplastic chromaffin cells. The rat pheochromocytoma cell line, PC12, has been used extensively in studies concerning NGF. PC12 cells respond to NGF treatment by an inhibition of proliferation and a stimulation of differentiation (175). The mechanisms controlling this response are presently unknown. The PC12 receptor has been defined in ligand-cross-linking studies as a single chain protein of 130 kDa, although a smaller receptor of 100 kDa, possibly a degradation product, is present (176). The receptor in A875 melanoma cells has been partially purified by affinity chromatography; again a 98-kDa species is present, although larger species of 138 and 190 kDa are present (177). It is not known how these multiple NGF receptor species correspond to the two receptor species defined by their apparent dissociation constants of 2 pM and 2 nM (178, 179). Recently, six cDNA clones representing mRNAs induced in PC12 cells by NGF have been isolated; one of the clones, VGF8a, encodes a 90-kDa protein the mRNA of which is induced more than 50-fold by NGF (180).

Colony-stimulating Factors (CSF-1, CSF-2, Multi-CSF). The soft agar colony assay developed by Metcalf and Johnson (181) has led to the identification of a number of factors, called CSFs, that regulate the growth and differentiation of hematopoietic precursor cells. In common with other tissue GFs, CSFs are synthesized at a large number of sites in the body and are active in the low pM level. These factors include CSF-1 [formerly called macrophage CSF (182)], CSF-2 [granulocyte-macrophage CSF (183)], and granulocyte CSF (184). Another factor called interleukin 3, IL-3, is active in stimulating colonies of mixed cell type (185) and has been dubbed multi-CSF. This factor goes by various names in the literature, reflecting its stimulation of growth and differentiation of a variety of cell types: P-cell-stimulating factor (186); mast cell-stimulatory factor (187); hematopoietin 2 (188); burst-promoting activity (189); and hematopoietic cell growth factor (190). Unlike most GFs which are purified on the basis of a cell growth bioassay, IL-3 was described and purified on the basis of its ability to induce an enzyme (20α-hydroxysteroid dehydrogenase) in mouse spleen T-lymphocytes (191). The activity of IL-3 (multi-CSF) includes the promotion of growth and differentiation of granulocytes, macrophages, and multipotential stem cells as well as colony formation from early erythroid, eosinophilic, megakaryocytic, and mast cell progenitors (192). Multi-CSF has been purified and partially sequenced (185); cDNA clones corresponding to both human and mouse multi-CSF have been isolated (187, 193).

CSF-1 has been purified to homogeneity from mouse L-cells (182) and human urine (194), and radioreceptor and radioimmune assays have been developed (195, 196). Native CSF-1 from mouse L-cells is a 65–80-kDa sialoglycoprotein composed of two possibly identical chains linked by disulfide bonds (194). The variation in size is due in large part to variable carbohydrate side chain modification; the polypeptide chain itself may account for only 15 kDa of the size of the reduced chain. The human gene encoding CSF-1 has now been cloned (197); sequence of the cDNA clone indicates a pre-proCSF-1 of 252 residues with a 32-residue leader peptide. The proCSF-1 peptide (224 residues) may be further processed to a 20-kDa form by proteolytic processing after residue 188. Incubation of bovine marrow adhesive cells with either CSF-1 or multi-CSF will induce up regulation of the number of CSF-1 receptors (198). It has recently been reported that the product of the c-*fms* proto-oncogene is the receptor for CSF-1 (24). The c-*fms* protein is a 170-kDa transmembrane glycoprotein which displays tyrosine kinase activity (199, 200). As in the case of the v-*erb*B gene and the EGF receptor, the v-*fms* gene may encode a truncated version of the CSF-1 receptor (199, 201). Unlike the EGF receptor case, however, the v-*fms* protein does not appear to be significantly truncated. Since the c-*fms* gene is located on human chromosome 5 (202), it is interesting to note that a deletion in the long arm of this chromosome in bone marrow cells is associated with a syndrome in which patients are predisposed to myeloid leukemia (203) or polycythemia vera (204); patients displaying this 5 q– marker are hemizygous for a deletion of chromosome 5 which does include the c-*fms* locus (205).

The macrophage-granulocyte factor, CSF-2, is a glycoprotein that has been purified from endotoxin-treated mouse lung (reviewed in Ref. 206). The factor has now been cloned from three species, mouse, gibbon ape, and humans. Both the human and gibbon ape CSF-2 cDNA clones encode a protein of 144 amino acid residues (207) which is thought to be cleaved to form a mature protein of 127 residues (14 kDa). Sequencing of the mouse cDNA clone (208) reveals substantial sequence homology at the amino acid level to the corresponding residues in human CSF-2; there is 54% amino acid homology between mouse and human CSF-2 (207, 209). CSF-2 has also been called neutrophil migration-inhibitory factor (210).

Much less is known about other colony-stimulating factors, although significant progress has been made in purification. The murine factor called granulocyte-CSF (211) has been purified to homogeneity and runs as a 24.5-kDa band on a sodium dodecyl sulfate-polyacrylamide gel (212). This factor is apparently distinct from the differentiation factor [D factor (213)] now purified to homogeneity as a 62-kDa band (214). This latter protein may be identical to MGI-2 (215) and differentiation-inducing factor (216). The D factor induces differentiation of the human promyelocytic leukemia cell line HL-60 (216); chemical treatment of HL-60 cells leads to an induction of the c-*fms* proto-oncogene (205) and thus presumably receptors for CSF-1. Although the evidence is yet fragmentary, the induction of CSF-1 receptors by another CSF (factor D) would certainly be in keeping with a model of hematopoietic cell differentiation involving the regulation of hematopoiesis mediated through a complex cascade of intercellular protein factor signals. None of these factors have yet been cloned.

Autocrine and Paracrine Stimulation in Cancer

Autologous production of a growth factor by a cell bearing receptors for that same factor could result in a growth advantage.

The implications of such autostimulation for the growth of transformed cells are readily apparent (217–219). Hypotheses invoking autostimulatory models have also been proposed for smooth muscle cells, human osteosarcoma cells, chemically transformed mouse fibroblasts, and T-cell leukemia involving IGF-1, PDGF, TGFβ, and IL-2, respectively. Testing of the autocrine model in these systems has led to a mixed result. In three of these cases, it has been possible to use an anti-GF antibody which inhibits binding of the GF to its receptor. The smooth muscle cells have been shown to produce an IGF-1-like molecule and monoclonal antibodies to IGF-1 inhibit proliferation in a defined culture system (220). In the osteosarcoma case, it has been possible to demonstrate production of PDGF and functional PDGF receptors in the cloned cell line U-2 OS, as well as significant inhibition of growth in the presence of a polyclonal PDGF antibody (221). Another circumstance in which specific cells have been shown to both produce and respond to the same factor is with TGFβ in chemically transformed fibroblasts (87, 88, 91), although TGFβ antibody inhibition experiments have not yet been performed due to the lack of high-affinity antibodies to TGFβ. Interestingly, the change in the chemically transformed cells relative to their nontransformed parents is a greatly increased sensitivity to the TGFβ produced by the cells (and present in serum) and not increased production of TGFβ (222).

In the T-lymphocyte system, evidence indicates that antigen-initiated IL-2-dependent T-cell growth occurs normally through both autocrine and paracrine mechanisms. T-helper cells both produce and respond to IL-2, whereas the majority of cytolytic and suppressor T-cells do not produce IL-2 but proliferate in response to IL-2 derived from helper T-cells (paracrine stimulation) (222, 223). The gibbon ape leukemia cell line MLA-144 provides an excellent model for autocrine growth regulation. MLA-144 cells both produce and respond to IL-2 (224); furthermore, an anti-IL-2 antibody strongly inhibits the growth of this cell line.[6] It has not been possible to extend the autocrine stimulatory observations, however, to human T-cell leukemias. Freshly isolated leukemic cells and cell lines established from children with T-cell acute lymphoblastic leukemia do not produce or respond to IL-2. On the other hand, cells and cell lines from patients with adult T-cell leukemia which is associated with human T-leukemia virus 1 express IL-2 receptors but do not produce IL-2 (225). It is not known whether this constitutive display of IL-2 receptors on virally infected cells could operate in the same fashion as v-*erb*B in virus-induced erythroblastic leukemias (see below).

The autocrine model may well be adequate to explain growth in soft agar and relative growth factor independence of chemically transformed fibroblasts, several instances of simian sarcoma virus transformation, the serum factor independence of osteosarcoma cells, and perhaps even the pseudomalignant behavior of normal placental trophoblast (70). A second pathway involving a paracrine model might be at least as likely an explanation of how growth factor production might operate in the development of cancer. GFs produced by cancer cells could stimulate proliferation of stromal cells (*e.g.*, fibroblasts and vascular cells), a necessary occurrence for the development of large tumors. Alternatively, stromal cells may produce GFs that stimulate cancer cells. Such a situation in which tumor components (*i.e.*, neoplastic and stromal cells) cross-feed each other with factors could explain, in some cases, why it has not been possible to grow presumptive malignant cells in culture (*e.g.*, certain carcinoma cells). In line with a paracrine regulatory model, one might propose that the transformed epithelial cells are dependent on factors produced by the nonimmortalized, nontransformed stromal cells found in the tumor which might be unable to survive in long-term cultures. A second explanation would involve the growth-inhibitory feature of TGFβ on epithelial cell growth (87). Because TGFβ is a component of serum (1), the routine culture of tumor explants in serum-containing media might inhibit the outgrowth of TGFβ-inhibited epithelial cells. This explanation is consistent with the observation that most epithelial cell lines tested exhibit some degree of inhibition by TGFβ (85–87, 103).

[6] K. A. Smith, personal communication.

Growth Factors, Oncogenes, and the Cellular Response

Dissection of the cellular events intervening between growth factor binding to cell surface receptors and the initiation of DNA synthesis is one of the major tasks of cell biology and cancer biology. The machinery that transduces the growth factor signal to the cell nucleus includes the growth factor receptors, their substrates, a number of key enzymes (including kinases and lipases), cytoskeletal proteins, transcriptional factors, DNA-binding proteins, and lastly a complex of enzymes which channel deoxy- and ribonucleotide precursors into the growing forks of DNA replication (226). Possible scenarios for GF induction of DNA synthesis and alterations in neoplastic transformation (see Fig. 1) might proceed as follows.

1. GF binds to its cognate cell surface receptor. In response

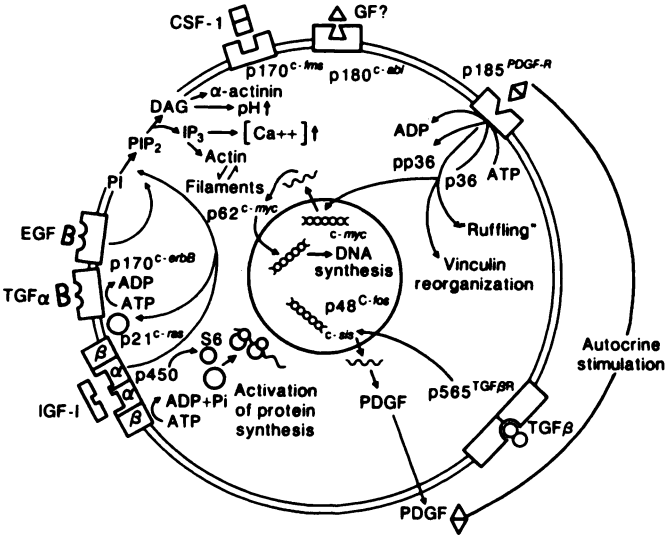

Fig. 1. Involvement of proto-oncogene cell products in the growth factor-receptor-response pathway. Specific high-affinity receptors (*R*) for GFs are indicated as *rectangles* in the plane of the cell membrane, each with its own specific site for GF binding; subunit structure is indicated. The c-*myc* and c-*sis* proto-oncogenes are indicated as double helices within the cell nucleus; their mRNA transcripts are indicated by a *single wavy line*. The phosphatidylinositol pathway (PI → PIP_2 → DAG + IP_3) is indicated as taking place in the plane of the membrane. The protein product of the c-*fos* oncogene is indicated in a nuclear compartment. Although this fictional cell is indicated to bear receptors for seven different GFs, the various GF receptors show a degree of cell type specificity (see Table 1). No attempt is made to indicate the process of receptor internalization and/or down regulation. See text for further explanation. *p170*, 170-kDa protein (other proteins are similarly designated); *pp36*, 36-kDa phosphoprotein.

to GF binding, the receptor may undergo an allosteric change, a redistribution in the membrane, or an association with other membrane proteins. The EGF receptor, for example, is a 170-kDa glycoprotein located at the cell surface, possessing an extracellular EGF binding domain, a transmembrane region, and a cytoplasmic face bearing domains which bind ATP and substrate(s) for phosphorylation (48). In the presence of EGF, the receptor density on the cell surface decreases ("down-regulation") as the GF-GF receptor complex is internalized into "receptosomes" (227).

2. The activated GF receptor activates a number of intracellular substrates. Although the EGF receptor can phosphorylate itself (49), it may also lead to the phosphorylation of a 35-kDa protein in a Ca^{2+}-dependent fashion (228), a 36-kDa protein (229–231), possibly a 42-kDa protein (232), or even phosphatidylinositol (233). Other possible targets for phosphorylation include vinculin (234) and the glycolytic enzymes enolase and phosphoglycerate mutase (235).

Activation of the receptor can sometimes occur in the absence of growth factor. Sequence homology between the *erb*B gene product and the cellular receptor for EGF (23) suggested that the chief feature distinguishing the two is the absence of the EGF-binding domain in the retroviral version, suggesting a mode of oncogene activation in which the *erb*B protein might function to relay a mitogenic signal even in the absence of ligand (EGF) binding. Recent evidence confirms this model in ALV-induced chicken erythroleukemias; every case analyzed in one study apparently involved the integration of an intact ALV genome into the c-*erb*B locus in a fashion that would lead to the overexpression of a truncated EGF receptor under the control of the introduced ALV promoter (236). In this way, the cell expressing a truncated GF receptor might be constitutively activated to a "turned-on" state regardless of the presence of the growth factor. Moreover, evidence has led to an identification of the protein product of the c-*fms* oncogene as the cell surface receptor for the hematopoietic stem cell growth factor CSF-1 (24); the v-*fms* oncogene may encode an altered form of the receptor.

EGF receptor gene (c-*erb*B) homology to other members of the *src* gene family has led to the speculation that one or more of these c-oncogenes may encode GF receptors (25). Some of the *src*-related proto-oncogenes may encode enzymes involved in the increased intracellular formation of inositol triphosphate and diacylglycerol (233). Although both the 35- and 36-kDa proteins are phosphorylated on tyrosines as well as serines and threonines, the significance of the tyrosine phosphorylation has not provided the key to growth control as had first been hoped.

3. The increased concentrations of inositol triphosphates and diacylglycerol is followed by a transient increase in cytosolic-free calcium, an activation of protein kinase C and adenyl cyclase, and a reorganization of the cytoskeleton. These middle early events occur within several min after GF stimulation; it is not known whether their temporal proximity reflects any causal relationship. However, recent evidence points to an interplay between diacylglycerol and the interaction of α-actinin with the cell membrane (237) and between phosphatidylinositol 4,5-bisphosphate and actin polymerization (238). PDGF induces a rapid reorganization of the cytoskeleton of human fibroblasts within 2 min marked by the formation of circular membrane ruffles within 15 min (239). In addition to its effects on cytoskeletal actin, PDGF also induces a redistribution of vinculin within minutes of treatment (240); TGFβ will induce cytoskeletal changes similar to those seen for PDGF.[7] Likewise, EGF will induce transient ruffling behavior and a long-term reorganization of A431 monolayer morphology (241).

Recent evidence indicates that the p21 product of the *ras* gene may be involved in growth factor signal transduction. Besides induction of morphological transformation (242, 243), microinjection of p21 induces DNA synthesis (244). Even more meaningful are the studies with microinjection of monoclonal antibodies to p21 (26). The antibodies block serum or EGF/insulin stimulation of DNA synthesis indicating that the *ras* p21 is an obligatory intermediate in the transduction of the growth factor stimulus. Further, it suggests another step at which a lesion may occur as a step in neoplastic transformation. If a postreceptor mechanism is constitutively activated, the cell may continuously receive a proliferative stimulus without the need for a growth factor or its receptor. Such may be the mechanism of transformation by activated *ras*.

4. GF stimulation of quiescent cells brings about transcriptional activation of a number of genes in the middle time frame (20 min–4 h). One of the most striking consequences of GF stimulation is the induction of c-oncogene transcription. Treatment of fibroblasts with PDGF brings about a 40-fold elevation of c-*myc* mRNA levels within 2 h (27) and a similar increase in c-*fos* mRNA levels within 45 min (28–30). Recent evidence using Chinese hamster lung fibroblasts, however, indicates that the increased accumulation of c-*myc* transcripts may be posttranscriptional (245). FGF and EGF share this ability to induce c-*fos* gene transcription (29). PDGF induces the c-*myc* gene in cultured placental trophoblast cells as well (70). Other genes induced by growth factors include β- and γ-actin by EGF (246) and three mRNAs of unknown function, KC, JE, and JC [related to c-*fos* (247)], after PDGF treatment (248). In addition, TGFβ induces a peak of actin mRNA the magnitude of which is TGFβ dose dependent (249) which correlates with the degree of morphological transformation apparent at 24 h after TGF treatment (43). As has been mentioned, TGFβ also induces the c-*sis* proto-oncogene in mouse fibroblasts, the translation product of which (a PDGF-related mitogen) is suggested to serve as the mitogen mediating the action of TGFβ on AKR-2B cells.[4] In this middle time frame, GF treatment brings about a 2- to 4-fold rise in the rate of protein synthesis, accompanied by the phosphorylation of ribosomal protein S6 (250).

5. Several GF-induced proteins are localized to the nucleus of stimulated cells and may be involved in the pleiotropic activation of growth-regulated genes. The products of the c-*myc* and c-*fos* genes (251, 252) are presumably DNA-binding proteins (253) found chiefly in the cell nucleus (29, 254, 255). The c-*fos*-encoded protein increases rapidly in concentration after PDGF stimulation and is found localized to the cell nucleus 1 h after stimulation (29). Similarly, an unidentified 29-kDa protein is rapidly induced by PDGF in BALB/c-3T3 cells and becomes localized in the nucleus (256). It appears that the level of c-*myc* gene expression correlates well with the level of proliferative activity in placental trophoblast (257) and the state of lymphocyte proliferation (258). These results would suggest that the c-*myc* product may reflect the cell's commitment to proliferation, perhaps through an activation of other growth-related genes.

[7] W. J. Pledger, personal communication.

Constitutive activation of growth factor-regulated genes such as c-*myc* in some circumstances results in an apparently continuous stimulus to proliferate. Such may be the case with certain B-cell tumors, such as mouse plasmacytoma and Burkitt's lymphoma, where derived cell lines all show characteristic chromosomal translocations involving the c-*myc* locus (259, 260). In these tumors, the chromosomal rearrangements presumably transcriptionally activate the c-*myc* locus, resulting in a high constitutive level of c-*myc* mRNA; it is this transcriptional activation of c-*myc*, it is argued, that drives their uncontrolled proliferation (261). The constitutive high-level synthesis of *myc* mRNA is not sufficient to transform fibroblastic cells, however. Transfection of primary rat fibroblasts with an activated *myc* gene is not sufficient to cause formation of transformed foci (262). Full transformation seems to require a second cooperating oncogene from the *ras* family (262). Armelin *et al*. (263) have helped to clarify this puzzling observation by transfecting 3T3 cells with a c-*myc* gene construct that allowed high-level continuous presence of c-*myc* mRNA in the presence of glucocorticoids. Instead of transforming the target cell, the c-*myc* gene activation led to an independence of the cells from PDGF stimulation (263).

The situation becomes more clear in light of the competence-progression model of Pledger *et al*. (9, 10) in which growth factors can be divided into two groups. Competence factors, such as PDGF, induce a state of "competence" to respond to a second GF signal which might come from stimulation by insulin-like growth factors or EGF [progression factors for BALB/c-3T3 cells (44)]. In light of the c-*myc* gene induction by PDGF (27), one might therefore speculate that expression of c-*myc* protein could be part of the state of competence (264). However, recent evidence suggests that c-*myc* gene induction is necessary, but not sufficient for induction of competence in normal human B-lymphocytes (265). One model might thus divide not only growth factors into competence and progression groups but their cellular targets as well. In this way, certain oncogene cell products may be involved in competence (*e.g., myc*, *myb*, E1a, *fos*, *sis*), others in progression (*e.g., ras, Blym, raf/mil*), and still others in both (polyoma middle T). If *myc* expression is a competence phenomenon (and not a growth phenomenon *per se*) reflecting exposure to GFs (70, 266), then it is not surprising to learn that cells grown in the presence of serum would show *myc* transcripts and *myc* protein regardless of their particular cell cycle phase (267).

Summary and Conclusions

Growth factors, defined as polypeptides that stimulate cell proliferation, are major growth-regulatory molecules for cells in culture and probably also for cells *in vivo*. Nontransformed cells show an absolute requirement for growth factors for proliferation in culture and generally more than one growth factor is required. Under usual culture conditions, growth factors are more rapidly

Table 1

Growth factor	Primary translation product	Mature factor size	Cell source	Target cell	Receptor	Ref.
EGF	1168 or 1217 aa[a]	6 kDa (53 aa)	Submaxillary gland, Brunner's gland, possibly parietal cells	Wide variety of epithelial and mesenchymal cells	c-*erb*B gene; 170 kDa tyrosine kinase	23, 31–41
TGFα	160 aa	5.6 kDa (50 aa)	Transformed cells, placenta, embryos	Same as EGF	Same as EGF	75–84
PDGF	241 aa (B chain); A chain unknown; B chain encoded in c-*sis* proto-oncogene	32 kDa (16 kDa B chain; 14–18-kDa A chain), + CHO	Blood platelets, endothelial cells, placenta	Mesenchymal cells, smooth muscle, placental trophoblast	185 kDa tyrosine kinase	55–57, 67–70, 73–74
TGFβ	391 aa	25 kDa (2 × 112 aa)	Blood platelets, kidney, placenta, cultured cells	Fibroblastic cells, keratinocytes, mammary epithelial cells, carcinoma, and melanoma lines	565–615 kDa complex (2 × 280–290 kDa)	85–99, 105–108
IGF-I	130 aa	7 kDa (70 aa)	Adult liver and other sites, smooth muscle cells	Epithelial, mesenchymal	450 kDa complex (2 α chains of 130 kDa; 2 β chains of 85 kDa)	123–134
IGF-II	180 aa	7 kDa (67 aa)	Fetal liver, placenta	Epithelial, mesenchymal	Single polypeptide chain of 260 kDa	123–134
IL-2	153 aa (mouse); 169 aa (human)	15 kDa (133 aa); some CHO	T-helper cells	Cytotoxic T-lymphocytes	55 kDa (33 kDa protein + 22 kDa CHO)	137–140, 143–147
FGF	Unknown	14–18 kDa (basic FGF is 146 aa)	Brain, pituitary, chondrosarcoma	Endothelial cells, fibroblasts	Unknown	149–165
β-NGF	307 aa	26 kDa (2 × 118 aa)	Submaxillary gland	Sympathetic and sensory neurons	130 kDa (possibly kinase)	170–179
CSF-1	252 aa	70 kDa (2 × 35 kDa); 60% CHO	Mouse L-cells	Macrophage progenitors	c-*fms* proto-oncogene; 170 kDa tyrosine kinase	24, 194–205
CSF-2 (granulocyte-macrophage CSF)	144 aa	15–28 kDa (127 aa) (1–50% CHO)	Endotoxin-induced lung; placenta	Macrophage and granulocyte progenitors	Unknown	183, 192, 206–210
Multi-CSF (IL-3)	144 aa	28 kDa (134 aa) (50% CHO)	T-lymphocytes	Eosinophil, mast cell, granulocyte, macrophage progenitors; T-lymphocytes	Unknown	185, 188, 190–192

[a] aa, amino acid residues; CHO, carbohydrates.

depleted than other media components and thus become rate limiting for proliferation. The loss of or decreased requirement for specific growth factors is a common occurrence in neoplastically transformed cells and may lead to a growth advantage, a cardinal feature of cancer cells. Recent work with transforming growth factors, the platelet-derived growth factor, and oncogenes has produced some insight into the mechanisms through which alterations in growth factor-receptor-response pathways could lead to a growth advantage. Evidence has been derived for autocrine secretion in which the cell produces its own growth factor. Many transformed mesenchymal cells produce PDGF (the product of the c-*sis* proto-oncogene) and certain transformed cells both produce and respond in a growth-stimulatory manner to TGFβ. With TGFβ, which is a growth inhibitor for certain epithelial and other cell types, the loss of the normal inhibitory response in transformed cells could have the same result as the activation of a growth-stimulatory response.

Two proto-oncogenes, *erb*B and *fms*, encode growth factor receptors. In the *erb*B case, the viral *erb*B aberrant receptor produced is truncated and appears to be constitutively activated without the need for a growth factor. Recent studies suggest that the p21 product of the *ras* oncogene may be an obligatory intermediate in transducing the growth factor signal. Activation of *ras* may, therefore, activate the growth factor pathway without the need for either a growth factor or its receptor. The transcription of *myc* and *fos* is induced by growth factor stimulation of quiescent cells. The protein products of both are nuclear associated and conceivably could be involved in regulating other genes important in the control of cell proliferation. Activation or inappropriate expression of either *myc* or *fos* could produce the same end result as stimulation of a growth factor pathway leading to a growth advantage.

Study of the molecular mechanism(s) of growth factor action has just begun. The excitement and attention focused on cellular oncogenes in recent years is now turning toward growth factors, not only as they concern the control of normal cell growth but also the involvement of growth factor-initiated pathways in the etiology of cancer.

One important implication of the molecular dissection of growth control is the identification of specific genes important in growth regulation. The genes encoding growth factors, growth factor receptors, and the post-receptor machinery (*i.e.*, the products of the *sis*, *erb*B, *fms*, *ras*, *fos*, *myb*, and *myc* proto-oncogenes as well as the p53 gene) may be a significant subset of these pivotal regulatory genes. The cell specificity of these genes (see Table 1) may imply that it would be possible to treat neoplastic diseases with a more targeted arsenal of therapeutic agents which focus their effects on a narrower range of proliferative cells than today's drugs with more generalized actions. In this way, an agent which might interfere with the TGFβ-*sis*-PDGF pathway might inhibit mainly mesenchymal cell proliferation in a sarcoma, leaving untouched the proliferation of normal cells in the hemopoietic lineage and the intestinal epithelium, so often a side effect of the current generation of chemotherapeutic agents.

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