Growth factor superfamilies and mammalian embryogenesis

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Summary

With the availability of amino acid and nucleotide sequence information has come the realization that growth factors can be clustered into superfamilies. Several of these superfamilies contain molecules that were not initially identified because of growth-promoting activities; rather they were discovered through their ability to regulate other processes. Certain members of these superfamilies are present during early mammalian embryogenesis. However, until recently, it has been difficult to manipulate the developing mammalian embryo to observe directly the effects of inappropriate, excessive, or reduced expression of these molecules. Despite this limitation, at least some of these molecules have been implicated in the control of differentiation and morphogenesis, two actions unpredicted from the cell biology of most of the growth factors. Moreover, these actions are reflected in nonmammalian species where homologues of the mammalian growth factors control crucial steps in the choice of developmental fate. This review describes five growth factor superfamilies and the role these molecules may have in controlling proliferation, differentiation, and morphogenesis during mammalian development.

Key words: growth factor, mammal, epidermal growth factor, EGF, insulin-like growth factor, IGF-I, IGF-II, transforming growth factor-beta, TGF, heparin-binding growth factor, HBGF, platelet-derived growth factor, PDGF.

Introduction

Recent analysis of early development of C. elegans, Drosophila, and Xenopus has emphasized the role that growth factors, or related molecules, may play as morphogenetic and differentiation signals. Two well-characterized growth factors have sequence similarity to pattern-forming genes. Transforming growth factor-beta is homologous to predicted proteins of the decapentaplegic complex of Drosophila and a maternally encoded mRNA in Xenopus embryos (Padgett et al. 1987; Weeks & Melton, 1987). The gene encoding epidermal growth factor shares domains of homology with the homeotic loci lin-12 in C. elegans and Notch in Drosophila (Greenwald et al. 1985; Wharton et al. 1985; Knust et al. 1987). Moreover, growth-factor-like molecules act as mesoderm-inducing signals in developing Xenopus blastocysts (Smith, J. 1987; Slack et al. 1987; Kimelman & Kirschner, 1987). Slack et al. (1987) and Kimelman & Kirschner (1987) showed that purified bovine basic fibroblast growth factor can induce mesoderm differentiation from ectoderm tissue. Thus, growth factors, or novel growth-factor-like molecules, seem to guide morphogenesis and differentiation in these species.

Can the same be said for mammals? An emerging body of literature suggests that polypeptide growth factors which are similar, if not identical, to those isolated from sera and tissues of adult mammals, influence not only growth but differentiation and morphogenesis during mammalian development. This view is furthered by the realization that several molecules originally identified because of their developmental roles are structurally similar to growth factors. As shown in Table 1, the expanding number of growth factors can be grouped into superfamilies based on nucleotide and amino acid sequence homology as well as similar receptor-binding activity. In Table 2 and the paragraphs below, we summarize the chemistry and biology of these superfamilies as well as the data that link them to mammalian development.
The epidermal growth factor family

Several structurally related peptides exhibit the activity first identified with epidermal growth factor (EGF). EGF was discovered by Stanley Cohen as a contaminant within certain nerve growth factor preparations which triggered premature eyelid opening and incisor eruption in neonatal mice (for review see Carpenter & Cohen, 1979). Using this observation as a bioassay, Cohen and his associates purified EGF from mouse salivary glands and, by amino acid analysis, showed it to be homologous to the human hormone urogastrone. A structurally and functionally similar growth factor, transforming growth factor-alpha (TGF-alpha), is produced by certain tumour and retrovirus-transformed cells (Marquardt et al. 1984). Finally, Brown et al. (1985) have described a vaccinia-virus-encoded mitogen which has EGF activity. All members of the EGF superfamily compete efficiently with salivary gland EGF for binding to a $170 \times 10^3\, M_r$ transmembrane tyrosine-specific kinase, which is the product of the c-erb B proto-oncogene (Downward et al. 1984).

Early embryological studies have focussed primarily on the effects of EGF, administered in utero, on the developing lung and palate (Catterton et al. 1979; Gentry 1987; Teixido 1987). Thus, these EGF-like factors may play a multifunctional role during development. Adamson & Meek (1984) demonstrated that, whereas the number of EGF receptors increases in fetal tissues, the apparent affinity for EGF declines two- to threefold during mouse gestation. The authors suggest that these changes correlate with differing roles of the receptor as development proceeds from tissue growth to differentiation. Thus, EGF may stimulate proliferation in stem cells and differentiation or expression of a differentiated phenotype in mature cells. This is supported by the range of nonproliferative responses to EGF seen in differentiated cells (for review see Sporn & Roberts, 1987). The homology between EGF and the homeotic loci Notch and lin-12, mentioned above, may imply a novel role for EGF and TGF-alpha. Like the Notch and lin-12 products, the EGF and TGF-alpha precursors are thought to encode transmembrane peptides (Rall et al. 1985; Gentry et al. 1987; Teixidó et al. 1987). Thus, these

<table>
<thead>
<tr>
<th>Group</th>
<th>Members</th>
</tr>
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<tbody>
<tr>
<td>Epidermal growth factor</td>
<td>EGF, Transforming growth factor-α, TGF-α, Vaccinia growth factor, VGF</td>
</tr>
<tr>
<td>Insulin-like growth factor</td>
<td>IGF-1, IGF-II (Somatomedin-C; multiplication stimulating activity, MSA)</td>
</tr>
<tr>
<td>Transforming-growth factor-β</td>
<td>TGF-β₁, TGF-β₂, Inhibin-A, Inhibin B, Activin-A, Activin AB</td>
</tr>
<tr>
<td>Heparin-binding growth factors</td>
<td>Acidic HBGF (acidic fibroblast growth factor, αFGF; endothelial cell growth factor, ECGF)</td>
</tr>
<tr>
<td>Platelet-derived growth factor</td>
<td>PDGF-A, PDGF-B (sis product), PDGF-AB</td>
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precursor molecules may act on the cell surface during development to mediate cell—cell interactions by recognizing a complementary receptor on another cell.

**Insulin-like growth factor family**

The insulin-like growth factors (IGFs) are a pair of growth factors with striking amino acid sequence and structural similarity to human proinsulin (for review see Blundell & Humbel, 1980). The IGFs have been referred to as sulphation factors, nonsuppressable insulin-like activity, multiplication stimulatory activity (MSA) and somatomedins. IGF-I is the more basic of the IGFs. IGF-II, the more neutral, is identical to the MSA molecule of rodents which has been well characterized and its gene cloned (Whitfield et al., 1984). The analysis of cDNA and genomic clones for human IGF-I and -II has shown that the IGF-II and insulin genes are contiguous and map to a nonallelic genes which encode human relaxin, a peptide secreted by the ovary and involved in the prenatal maturation of the female reproductive tract (Crawford et al. 1984). IGFs bind to at least two types of IGF receptors, as well as the insulin receptor, with different affinities (King & Kahn, 1985). In plasma, circulating IGFs bind specifically to carrier proteins which may modulate activity (Zapf et al. 1975).

Midgestation human and rodent tissues express IGFs (Table 2). IGF-II mRNA and protein levels predominate during gestation and decline within a few weeks after birth. IGF-I, however, exhibits the opposite pattern of expression. From these results, it is generally assumed that IGF-II is primarily a fetal mitogen involved in the development of embryonal tissues. In support of this conclusion is the secretion of IGF-II by differentiated embryonal carcinoma lines (Nagarajan et al. 1985; Heath & Shi, 1986).

One exception to the pattern of IGF-II expression in fetal development is noted in neural tissue. High levels of IGF-II protein, mRNA and receptors are seen in the adult brain where IGF-II may stimulate neural outgrowth (Sara et al. 1982; Recio-Pinto & Ishii, 1984; Haselbacher et al. 1985).

As shown in Table 2, receptors and binding proteins for the IGFs have been detected at various stages of mouse embryogenesis. The presence of IGF-binding proteins in amniotic fluid and blastocysts suggests the early action of IGFs. PC13 embryonal carcinoma cells express IGF receptors and secrete binding protein into the medium (Heath & Shi, 1986; Smith et al. 1987). Upon differentiation of these cells in response to retinoic acid, IGF-II receptor expression declines while IGF-II and binding protein synthesis increase. Since PC13 cells respond to IGF-II, Heath and co-workers proposed that these cells and their embryonic analogues are under paracrine and, following differentiation, autocrine control by IGF-II.

**Table 2. Examples of growth factor expression during embryogenesis**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Species</th>
<th>Material assayed</th>
<th>Occurrence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-α</td>
<td>Mouse</td>
<td>TGF-α mRNA</td>
<td>Post-day-7 embryos</td>
<td>Proper et al. 1982; Twardzik, 1985</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>TGF-α mRNA</td>
<td>Post-day-7 embryos</td>
<td>Poppelier et al. 1987</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>TGF-α mRNA</td>
<td>Post-day-8 embryos</td>
<td>Lee et al. 1985</td>
</tr>
<tr>
<td>EGF Receptor</td>
<td>Mouse</td>
<td>EGF binding</td>
<td>Post-day-11 embryos</td>
<td>Nexo et al. 1980; Adamson et al. 1981</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Receptor kinase</td>
<td>Post-day-10 embryos</td>
<td>Hortsch et al. 1983</td>
</tr>
<tr>
<td>IGF</td>
<td>Rat</td>
<td>Serum IGF-II</td>
<td>Fetal level &gt; maternal level</td>
<td>Moses 1980</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Serum IGF-I</td>
<td>Maternal level &gt; fetal level</td>
<td>D’Ercol et al. 1980; Sara et al. 1980</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>IGF-II mRNA</td>
<td>Amnion/ylk sac mesoderm</td>
<td>Heath &amp; Shi, 1985</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>IGF-I, II mRNA</td>
<td>Post-day-14 embryos/adult</td>
<td>Lund et al. 1986</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>IGF-I, II mRNA</td>
<td>Predominant expression in connective and mesenchyme-derived tissue</td>
<td>Han et al. 1987</td>
</tr>
<tr>
<td>IGF Receptor</td>
<td>Mouse</td>
<td>IGF-I, II receptors</td>
<td>Day-9, -12 embryos</td>
<td>Smith et al. 1987</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>IGF-binding proteins</td>
<td>Blastocysts/day-9 embryos</td>
<td>Smith et al. 1987</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>IGF-binding</td>
<td>Amnion</td>
<td>Drop et al. 1984</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Mouse</td>
<td>TGF-β mRNA</td>
<td>Day-17 embryo</td>
<td>Proper 1982</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>TGF-β mRNA</td>
<td>Day-21 fetal calvariae</td>
<td>Pleilshifter &amp; Mundy, 1987</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>TGF-β mRNA</td>
<td>Day-12, -13 embryos</td>
<td>Twardzik et al. 1982</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>TGF-β mRNA</td>
<td>Placenta</td>
<td>Frolick et al. 1983</td>
</tr>
<tr>
<td>HBGF</td>
<td>Mouse</td>
<td>int-2 RNA</td>
<td>Day-7, -8-5 embryos</td>
<td>Jakobovits et al. 1986</td>
</tr>
</tbody>
</table>
The transforming growth factor-beta family

Transforming growth factor beta (TGF-beta) is a dimer of two \(12.5 \times 10^3 M\), peptides expressed in a wide array of cell types. TGF-beta was originally identified by its ability to induce anchorage-independent growth of rat fibroblasts in conjunction with TGF-alpha or EGF (for review see Sporn et al. 1986; Massagué, 1987). TGF-beta cDNA clones of the human gene were obtained in 1985 (Derynck et al. 1985). More recently, additional TGF-beta-like genes and proteins have been described. These include two new forms of TGF-beta, the Müllerian-inhibiting substance (MIS), and the beta subunits of the inhibitors and activators of follicle-stimulating hormone secretion (Cheifetz et al. 1987; Mason et al. 1985; Vale et al. 1986; Ling et al. 1986; Cate et al. 1986).

TGF-beta best illustrates the multiplicity of responses elicited by growth factor action. Depending on the cell type and on other growth factors acting on the cell, TGF-beta may either promote or inhibit cell division in culture (Roberts et al. 1985). Differentiation may also be positively or negatively controlled. In cell culture, TGF-beta induces chondrogenesis and squamous differentiation of bronchial epithelial cells while myogenesis and adipogenesis are inhibited (for review see Sporn et al. 1986).

The TGF-beta-related protein, MIS, is produced by the developing testis and is responsible for the regression of the Müllerian duct. Expression of cloned human and bovine MIS in vitro yields a peptide capable of inducing regression of the rat Müllerian duct in organ culture (Cate et al. 1986). Studies of growth factor expression suggest that TGF-beta itself plays a role during embryogenesis (Table 2). TGF-beta activity is present in human placenta and in mid- to late-gestation mouse and rat embryos. Also, conditioned culture media of rat fetal calvariae explants contains TGF-beta activity. In these cultures, osteotrophic hormones that increase bone resorption also increase TGF-beta production. Since TGF-beta is mitogenic for osteoblasts, these results suggest that TGF-beta links resorption to subsequent proliferation and differentiation of osteoblasts.

Little is known about the ontogeny of TGF-beta receptors during development. Chieftetz et al. (1987) demonstrated that three types of TGF-beta receptors bind the different forms of TGF-beta with different affinities, thus defining a complex pattern of ligand–receptor interactions reminiscent of the IGF system. TGF-beta receptors are found on essentially all cells. If the receptors are also widespread in the early embryo, it will be important to determine whether TGF-beta exerts its influence locally or systemically.

The heparin-binding growth factor family

The heparin-binding growth factors are responsible for the activities ascribed to nearly a dozen previously identified factors (Lobb et al. 1986). The growth factor activities formerly known as acidic fibroblast growth factor, endothelial cell growth factor, eye-derived growth factor-II, retina-derived growth factor-I and anionic hypothalamus-derived growth factor are either identical to or are enzymatically processed derivatives of acidic HBGF. Likewise, basic HBGF is identical or related to basic fibroblast growth factor, eye-derived growth factor-I, retina-derived growth factor-II, cationic hypothalamus-derived growth factor-II and macrophage-derived growth factor. The genes encoding the acidic and basic HBGFs have been isolated as cDNA clones (Abraham et al. 1986; Jaye et al. 1986). Together, the HBGFs probably account for much of the activities associated with tumour angiogenesis (see review by Folkman & Klagsbrun, 1987). In addition, three oncogenes (int-2, hst, and a gene isolated from Kaposi's sarcoma DNA) encode proteins which are members of the HBGF family (Dickson & Gordon, 1987; Taira et al. 1987; Delli Bovi et al. 1987).

Recent work suggests that the HBGFs participate in early mesoderm development. Mouse embryonal carcinoma cells produce HBGF-like molecules known as embryonal carcinoma-derived growth factors (ECDGFs) (Heath & Isacke, 1984; van Veggel et al. 1987). The ECDGFs are mitogenic for mesoderm-like, but not endoderm- or ectoderm-like, derivatives of PC13 cells. In experiments designed to induce mesoderm differentiation from animal pole ectoderm of Xenopus blastocysts, Slack et al. (1987) showed that ECDGF and a purified HBGF, basic fibroblast growth factor, could mimic a component of the vegetal mesoderm-inducing signal. These results suggest a role for HBGFs in promoting the development of early mesoderm in mammals as well as in Xenopus. In addition, the HBGFs could conceivably promote angiogenesis during mammalian development. Indirect support for such a role is found in developing chicks where an angiogenic factor similar to basic HGBF has been isolated from neural tissue (Risau, 1986).

The platelet-derived growth factor family

Platelet-derived growth factor (PDGF) is a potent mitogen for cells of connective tissue origin and is thought to play a role in wound healing when released from platelets at the site of injury (for review see Ross et al. 1986). Purified PDGF from human platelets yields two distinct polypeptide chains, A and B, upon reduction. The amino acid sequences of the A and B
chains share greater than 50% positional identity. The A and B chains are encoded by separate genes which map to human chromosomes 7 and 22, respectively. A and B homodimers are mitogenic for fibroblast cells and either homodimer will compete for native PDGF isolated from human platelets in receptor binding assays. A dimeric structure linked by disulphide bridges is absolutely essential for PDGF mitogenic activity. PDGF within human platelets may consist of A:B heterodimers either in addition to, or in lieu of, A:A and B:B homodimers. Consistent with the mitogenicity of homodimers, the oncogene of simian sarcoma virus (v-sis) directs the synthesis of a PDGF B:B homologue.

Most developmental work involving PDGF has relied upon embryonal carcinoma cell lines. PDGF activity is detected in conditioned media from undifferentiated embryonal carcinoma lines (Gudas et al. 1983; Rizzino & Bowen-Pope, 1985). Retinoic-acid-induced differentiation greatly reduces the level of PDGF activity. While the PDGF secreted by these lines is not identical to PDGF from platelets, the embryonal carcinoma cell data imply the action of a PDGF-like factor during early embryogenesis. Consistent with this idea, transcripts of the A and B (c-sis) chain genes are seen in mouse embryos at gastrulation (M.M. & C.D.S., unpublished observations).

PDGF receptors are present on embryonal carcinoma cells. Rizzino & Bowen-Pope (1985) detected PDGF binding to retinoic-acid-differentiated F9 and PC13 cells and to two endoderm-like differentiated cell lines. In contrast, Gudas et al. (1983) did not see significant binding to retinoic-acid-treated F9 or PSA-1 cells, although the differentiated line PSA-1-G did exhibit receptors. No binding is seen in undifferentiated cells; however, it is plausible that the receptors are downregulated by endogenously produced factors.

**Intracellular mediators of growth factor action**

Some of the results cited above indicate that growth factors regulate differentiation and morphogenesis. Thus, it is important to understand how a peptide, such as HBGF, can function as a mitogen in one context and promote differentiation in another. Recent data indicate that growth factors, like other hormones, regulate gene expression within their target cells. Two classes of genes are induced: (1) common genes which seem to be general components of a growth factor response and (2) genes that are characteristic of a cell lineage. The first class of genes may be intracellular mediators of the growth factor response and may themselves influence gene expression, while the second class may dictate, in part, the nature of the response.

The initial event in growth factor action is binding to a specific, high-affinity receptor which spans the outer cell membrane. The receptor is often, but not always, a tyrosine-specific protein kinase (for review of kinases, see Hunter, 1987). The receptors for EGF (Ullrich et al. 1984; Lin et al. 1984; Xu et al. 1984), PDGF (Yarden et al. 1986), insulin (Ullrich et al. 1985; Ebina et al. 1985), and IGF-I (Ullrich et al. 1986) are tyrosine-specific kinases. The IGF-II receptor does not have tyrosine-specific kinase activity (Morgan et al. 1987). The receptors for TGF-beta and the HBGFs have not been characterized definitively. The current consensus holds that the binding of a growth factor by its receptor induces soluble, intracellular second messengers which transmit a signal to the nucleus (see review by Rozengurt, 1986). These second messengers may be phosphoproteins, inositol phosphates, diacylglycerol, cyclic nucleotides, monovalent or divalent ions. Within minutes after formation of the growth factor–receptor complex, changes in gene expression can be detected. Growth-factor-inducible genes have been described for PDGF (Cochran et al. 1983; Linzer & Nathans, 1983), EGF (Foster et al. 1982) and the IGFRs (Zumstein & Stiles, 1987), and many of these genes have been isolated as cDNA clones.

Some growth-factor-inducible genes appear to be lineage specific. For example, lymphocyte mitogens stimulate expression of the genes for interleukin-2, the interleukin-2 receptor and gamma interferon in resting T-cells (Kronke et al. 1985; Reed et al. 1986), but not in fibroblasts (C.D.S., unpublished observations). These tissue-specific genes dictate, in part, the differentiated functions of T-cells. In addition, there appears to be a cohort of genes which are expressed across tissue boundaries in response to numerous growth factors. This set of common genes includes the oncogenes c-myc and c-fos. The protein products of both c-myc and c-fos are associated with the cell nucleus and may regulate the expression of other genes. It is possible that c-myc and c-fos are prototypes of a class of genes whose products behave as intracellular mediators of the growth factor response (Armelin et al. 1984).

The influence of the cell's genetic programming in determining the cellular response to c-myc and c-fos expression is seen in the comparison of the different responses of PC12 and fibroblast cells following growth factor stimulation. The PC12 cell line is derived from a well-differentiated rat pheochromocytoma (Greene & Tischler, 1976). These cells proliferate rapidly and do not display differentiated markers until exposure to nerve growth factor (NGF). Contact with NGF causes PC12 cells to growth arrest and assume a neuronal phenotype.
Cessation of cell division and the onset of differentiation coincides with the induction of the c-myc and c-fos genes (Greenberg et al. 1985). The response of PC12 cells to NGF contrasts that of fibroblast cells which, following growth factor stimulation, express c-myc (Kelly et al. 1983) and c-fos (Greenberg & Ziff, 1984; Cochran et al. 1984; Kurolj et al. 1984; Muller et al. 1984) prior to cell division. Likewise, expression of activated src or ras genes in PC12 cells triggers growth arrest and cell differentiation, whereas the same genes promote uncontrolled cell division in fibroblast cultures (Alema et al. 1985; Bar-Sagi & Feramisco, 1985). In these cases, the differentiated status of the cell seems to predetermined the response to c-myc and c-fos.

Thus, different cell types may use the same switch mechanisms to stimulate different responses. This may explain the pleiotropic effects of growth factors such as the HBGFs, TGF-beta and EGF. Perhaps this interpretation can also address the similarities between growth factors and other molecules not known for growth factor activity. In addition to those included in Table 1, this group would include some products of pattern-forming and developmentally regulated genes of Drosophila, C. elegans, and Xenopus. The sequence and, in some cases, functional similarities between these molecules suggest a common evolution pathway for a variety of signalling factors, growth factors as well as other agents. Thus, the homology between Notch in Drosophila, lin-12 in C. elegans, and the EGF precursor may reflect a conserved signalling scheme.

Is only the signal conserved, or is cell cycle control a component of differentiation control? In cell culture models of terminal differentiation, such as embryonal carcinoma cell lines and PC12 cells, growth seems opposed to differentiation. However, in the embryo, where differentiation and proliferation coincide, the relationship is not so demarcated. Nonetheless, differentiation of the embryonic/abembryonic and the inner/outer axes of the mouse blastocyst does appear to be linked to cell cycle control (Kelly et al. 1978; Garbutt et al. 1987a,b). It is possible, therefore, that the control of some differentiation events are based on the cell's decision either to divide or not. Thus, a growth factor may control a differentiation event simply by governing cell division.

References


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