MOLECULAR AND CELLULAR APPROACHES TO THE CONTROL OF PROLIFERATION AND DIFFERENTIATION

Edited by

Gary S. Stein
and
Jane B. Lian

CELL BIOLOGY

A Series of Monographs

Molecular and Cellular Approaches to the Control of Proliferation and Differentiation

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Preface

For more than a century, it has been acknowledged that proliferation and differentiation are fundamental biological processes. Equally important, it has been understood that a relationship between cell growth and expression of phenotypic properties characteristic of specialized cells and tissues is associated with key regulatory events in the control of development as well as tissue repair. However, until recently, proliferation and differentiation were experimentally addressed independently.

It would be arbitrary and less than accurate to invoke any single explanation for the convergence of both concepts and experimental approaches that have provided the basis for addressing the integrated relationship between proliferation and differentiation. Indeed, the advances that have been made in molecular biology have played an important role in this context, permitting the assessment of a broad spectrum of biological parameters in single cells and tissue preparations and facilitating identification of cell growth and tissue-specific genes and their regulatory complexes (transcription factors and cognate regulatory elements). But it appears that it has been the combined application of molecular, biochemical, and morphological approaches, together with the development of *in vitro* systems that support differentiation and tissue organization, that has led to significant increments in our ability to define the proliferation–differentiation relationship.

To attempt coverage of all aspects of cell growth and differentiation in a single volume would be unrealistic and, at best, treatment of the principal elements of the developmental process and their control would be descriptive and superficial. Rather, in this volume we restrict our considerations to basic mechanisms involved in cell growth control, emphasizing the coupling of proliferation and the progressive expression of several specific cellular phenotypes. The manner in which cell structure is involved in the selective expression of genes associated with proliferation and differentiation and, in turn, how expression of such genes in response modulates both intracellular (nuclear matrix and cytoskeleton) and extracellular (extracellular matrix) architecture are emerging concepts that are addressed.

Most authors have focused primarily on a single model system or cell phenotype. But collectively these chapters provide information for beginning to assess the extent to which common signaling mechanisms and regulatory events are operative in the control of proliferation and differentiation in general. And while it would be premature to propose unifying mechanisms to explain the relationship of growth to differentiation, optimistically, the next few years should yield valuable insight into the regulation of this relationship as it is operative during early development and in the maintenance of structural and functional integrity of cells and tissues.

Gary S. Stein
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Regulation of Cell Proliferation

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Growth Factors: Their Role in the Control of Cell Proliferation

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Polypeptide growth factors act in a synergistic and sequential manner to promote the proliferation of nontransformed cells in culture. Although the mechanisms by which these factors impart mitogenic information to target cells are incompletely understood, recent studies have defined a series of biochemical and molecular events that occur in response to growth-factor treatment and as cells shift from a quiescent to a proliferative state. To initiate the mitogenic response, growth factors interact with, and consequently activate, specific membrane-bound receptors. Receptor activation, in turn, stimulates the formation of *second messengers,* which transduce the mitogenic signal from the cell membrane to the cell interior. As described below, the receptors for growth factors such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) possess an intrinsic ligand-activated tyrosine kinase; accumulating evidence suggests that it is via this activity

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that these receptors communicate with second messengers. Second messengers participate in a variety of events including, for example, the modification of transactivating factors that, via interaction with DNA response elements, induce the expression of specific genes. Proteins preferentially synthesized as a result of second messenger-mediated gene transcription modulate a host of regulatory processes that lead ultimately to the proliferative response.

Early studies identified the pre-DNA synthetic (G_1) phase of the cell cycle as the primary site of growth-factor action. Using cells arrested in early G_1 (G_0, see below) by mitogen deprivation, numerous investigators characterized events that occurred rapidly in response to growth-factor treatment. While the importance of these early G_1 responses is not to be minimized, data from other studies indicate that growth factor-dependent processes occurring in mid and late G_1 are also essential for proliferation. Thus, the purpose of this chapter is twofold: first, to describe potential mechanisms involved in growth factor-induced receptor activation and second messenger formation and, second, to detail changes in gene expression and other activities that occur throughout the G_1 phase of the cell cycle. Of the numerous growth factors previously characterized, PDGF and EGF have been extensively studied as models for growth-factor action in fibroblast systems. For this reason, and as a comprehensive review of all growth factors is beyond the scope of this chapter, we focus primarily on actions of these factors in fibroblastic growth control

I. GROWTH FACTORS AND RECEPTORS

Polypeptide growth factors form part of a large class of hydrophilic, extracellular signaling molecules, which constitute an important part of the endocrine system. Like classic peptide hormones, they bind to specific receptor proteins on the surface of target cells and regulate a wide variety of cellular functions through activation of several intracellular signals (discussed below). It has been useful to consider growth factors as a distinct class in that, unlike classic hormones, they are also important local mediators for cell regulation. Additionally, individual growth factors may be expressed in a wide variety of cells and can exhibit activity in a number of different target cells and tissues.

Molecular cloning techniques have greatly facilitated the identification of a growing number of growth factors, along with their complementary receptors. Three important themes have become apparent with further characterization of the role of growth factors and receptors in cell regulation. First, sequence and structural comparisons have allowed both growth factors and receptors to be recognized as members of distinct families (see discussion on receptors). Second, strong evidence has been found that, surprisingly, common mechanisms are shared between the different families both for receptor activation and for how the extra-

cellular signal is then subsequently conveyed through activation of intersecting intracellular signaling pathways. This will be discussed in part in this section, in terms of the common structural and functional aspects of the different receptors, and later in terms of the intracellular events following activation. Last, and probably most relevant to the functional effect of growth factors, is the understanding that cell proliferation and physiological responses are not specifically regulated by any one growth factor but are instead under the coordinate control of several factors acting at numerous stages in the growth and development of cells and tissues.

A. Fibroblast Proliferation Is Coordinately Regulated by Multiple Growth Factors

In vitro fibroblast systems have been extremely useful in the development of experimental paradigms for growth-factor action. The isolation of PDGF was prompted by the observation that fibroblasts could proliferate in growth medium containing serum but not platelet-poor plasma, the liquid fraction of unclotted blood. PDGF was identified as the primary factor among several released from platelet secretory granules that enabled fibroblasts to proliferate. This system was important in providing evidence that clearly demonstrated that both the concerted and sequential action of several growth factors was required in order to signal cells to divide.

The cell cycle can be defined as the sequence of events occurring from the completion of mitosis in the parent cell until the completion of the subsequent mitosis in one or both daughter cells.[1] In most cell systems, the cycle is made up of sequential phases consisting of the mitotic or M phase, the presynthetic gap or G_1 phase, the DNA-synthetic or S phase and the postsynthetic or G_2 phase. The majority of cells *in vivo,* however, are not cycling, but remain in a nonproliferating state during most of their life. Similarly, cells in culture may remain viable for an extended period in a growth-arrested or quiescent state referred to as G_0. Nontransformed fibroblastic cells, such as BALB/c 3T3 cells, may be growth-arrested either by growth-factor deprivation or by growth to a confluent density. The differences between noncycling, quiescent cells and those in a proliferating population have been an intriguing area of study, and the reader is referred to several excellent reviews for more detail.[1–3] Quiescent fibroblasts may be stimulated to reenter the cell cycle by exposure to several mitogenic factors. These have been termed competence factors because exposure to these factors alone is both required and sufficient to render the cells *competent* or responsive to additional factors in plasma that govern the further transition through the cell cycle and initiation of DNA synthesis[4] (see below). Studies employing BALB/c 3T3 cells identified PDGF as the primary competence factor present in serum,[5] although

several other factors including fibroblast growth factor (FGF), calcium phosphate crystals, and bombesin, have been subsequently identified as additional competence factors in either BALB/c 3T3 cells or other fibroblastic systems.[6–8]

PDGF is an approximately 30-kDa cationic glycoprotein composed of A and B polypeptide chains, which are encoded by two distinct homologous genes.[9] PDGF in its active form exists as either a disulfide-bonded homodimer or heterodimer of its two chains. PDGF, like most other peptide growth factors, is biologically active at nano- and picomolar concentrations, and interacts with its target cells by binding to a cell-surface receptor that exhibits both high affinity and selectivity for its ligand. The PDGF receptor shares several important common characteristics with other growth-factor receptors, which will be discussed in more detail below. Ligand binding results in rapid activation of receptor tyrosine kinase activity and, subsequently, changes in a variety of cell processes, including redistribution of vinculin and actin,[10] formation of inositol phosphates and consequent calcium mobilization (see below), cellular alkalinization,[11] and the induction of a number of early genes, including the cellular protooncogenes c-*fos* and c-*myc*.[12,13]

It is still unclear which events are actually required for the transition from a quiescent to a proliferative state. The use of mutant receptor constructs has shown that receptor kinase activity is crucial for the mitogenic function of the PDGF receptor[14] and, as will be discussed, for other growth-factor receptors as well.[15] The ability of antisense c-*myc* and c-*fos* oligonucleotides to inhibit DNA synthesis suggests that expression of *myc* and *fos* proteins is also required for mitogensis.[16,17] However, it is difficult to determine from these studies whether these proteins are required exclusively in the initial stage of the mitogenic response, or whether they might also function in regulating progression throughout the cell cycle. In BALB/c 3T3 cells, PDGF stimulation is not itself sufficient to promote a complete mitogenic response, which requires the subsequent and continuous exposure of PDGF-stimulated cells to progression factors found in plasma.[18] Importantly, cells exposed first to plasma and then to PDGF do not progress though the cell cycle, implying that not only may cell proliferation be coordinately regulated by multiple growth factors, but also that the regulatory events occur in a definite sequential order.

Progression factors include insulin-like growth factor 1 (IGF-1), a 7-kDa member of the insulin peptide family first identified as a mediator of growth hormone action,[19] and possibly EGF, a 6-kDa polypeptide isolated as an inducer of precocious eyelid opening and tooth eruption in newborn mice.[20] The specific function of these factors clearly depends on both the target-cell type and the context in which a particular cell is exposed to these factors. The effects of specific growth factors in other cell systems are not always so easily distinguished as in the BALB/c 3T3 system. For example, in C3H10T½ fibroblasts, EGF appears able to substitute for both competence and progression factors, and to act alone as a mitogen, albeit at concentrations significantly above those normally found in

serum. However, exposure to PDGF increases the sensitivity of these cells to EGF by more than 10-fold.[21] Thus, the synergistic effect of multiple growth factors may indeed be required to achieve an optimal mitogenic response at growth-factor concentrations normally encountered by cells.

The sequential regulation of mitogenic events by multiple factors observed in the 3T3 system is not restricted to fibroblastic cells. The proliferation of T lymphocytes is similarly regulated in discrete steps. In this system, either plant lectins (Con A or phytohemagglutinin), phorbol esters, or antigens play the role of competence factors in mitogenically activating quiescent cells. Exposure to these factors results in similar activation of intracellular responses and the induction of several of the same early genes as in BALB/c 3T3 cells.[22,23] Treatment with these factors alone is not sufficient to induce DNA synthesis, but enables cells to respond to interleukin 2 (IL-2) through up-regulation of the IL-2 receptor[24]; analogously, PDGF has been shown to up-regulate the IGF-1 receptor in 3T3 cells.[25]

The coordinate regulation of cellular events by multiple growth factors is important in a broader scope, apart from proliferation alone. In fact, in almost all cases, the differentiation and clonal expansion of cell populations from small numbers of stem cells depends on the action of multiple growth factors acting at discrete points during the expansion of the resulting cell lineages. In an elegant *in vitro* model, Zezulak and Green[26] demonstrated that growth hormone regulates two steps in the differentiation of an adipogenic fibroblast line. Growth hormone both promoted differentiation of the cells to preadipocytes, which then became responsive to IGF-I, and served to regulate the clonal expansion of these cells by inducing IGF-I synthesis in the differentiated cells. Optimal formation of differentiated hematopoietic colonies from *in vitro* bone marrow stem-cell cultures has been shown to require the synergistic action of IL-1 and at least one other hematopoietic growth factor.[27] *In vivo,* IL-1 was proposed to play a dual role during hematopoiesis by both stimulating the proliferation of quiescent stem cells and indirectly regulating their differentiation by subsequently up-regulating receptors for various hematopoietic factors in the stimulated cells.[28] Evidence that IL-1 can also induce production of hematopoietic growth factors themselves in a variety of stromal cells[29] illustrates how the control of differentiation may require a complex cascade of growth-factor interactions among several different cell types.

Several important roles for growth factors beyond the scope of this review should be mentioned. The chemotactic activities of several growth factors including PDGF,[30] fibroblast growth factor (FGF),[31] and transforming growth factor β (TGFβ)[32] are central to angiogenesis, wound healing, and tissue development. Several growth factors have been demonstrated to modulate the synthesis of extracellular matrix proteins, and their cellular receptors.[33,34] In light of growing evidence that the extracellular environment at least in part directs cellular respon-

siveness to growth factors, this provides an indirect pathway for growth control. Probably the widest role assumed by the TGFβ family of peptides, however, is their function as potent growth inhibitors, especially in epithelial and immune cells. The reader is referred to several recent comprehensive reviews for information on this important polypeptide family.[35,36]

B. Growth-Factor Receptors

Almost all of the receptors for polypeptide growth factors characterized have been identified as tyrosine kinases, and share a consistent set of structural and functional features. These receptors each contain three distinct structural regions: an extracellular ligand-binding domain, a hydrophobic domain that makes one pass through the cell membrane, and a hydrophilic intracellular domain containing a highly conserved kinase domain, in which resides the receptor tyrosine kinase activity. A more detailed comparison of structural characteristics has allowed several of the receptors to be placed in distinct families. The insulin-receptor family is characterized by a heterotetrameric receptor formed from two α and two β subunits, which are processed from a precursor molecule encoded by a single gene.[37–39] Two nearly identical insulin receptors and the IGF-I receptor share significant homology in the extracellular binding domain, which contains a single cysteine-rich region, and exhibits numerous conserved cysteine residues, glycosylation, and precursor cleavage sites. Members of the PDGF-receptor family possess extracellular ligand-binding regions characterized by multiple immunoglobulin-like domains, a lack of cysteine-rich regions, and a number of conserved cysteine residues and glycosylation sites.[40] A unique feature of this receptor family is that the conserved tyrosine kinase domain is split into two regions around a short, poorly conserved sequence of approximately 100 amino acids.[41] Members of this family include the receptors for PDGF (termed α and β), colony-stimulating factor 1 (CSF-1),[42] and the protein product of the c-*kit* gene,[43] recently identified as the receptor for stem-cell factor.[44] The FGF-receptor family bears some resemblance to the PDGF-receptor family but has a shorter ligand-binding domain.[15] A third receptor family includes the EGF receptor and the HER-2/neu receptor identified in rat cells.[45,46] In contrast to the insulin-receptor family, the extracellular portion of these receptors contains two cysteine-rich regions, which flank the putative ligand-binding site.[47] Several other receptors, including the nerve growth factor (NGF) receptor[48] and the IGF-II/mannose 6-phosphate receptor[49] appear unrelated to the above families and to each other.

The usefulness of these structural comparisons has been validated by evidence that similar receptors may be functionally related as well, in terms of overlapping specificities for binding several related growth-factor ligands. For example, high levels of insulin can activate the IGF-I receptor,[50] and there is good evidence that