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Views and Reviews

Cytoarchitecture and Cell Growth Control

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INTRODUCTION

Normal cellular growth control is a highly regulated process that responds to environmental cues provided largely by serum growth factors (SGF) and the extracellular matrix (ECM). SGF stimulation of substrate adherent, non-cycling or quiescent (G_0) cells promotes entry into proliferative phase growth. Such "activation" is accompanied by changes in gene expression [induction of immediate early response (IER) genes] and cytoarchitectural re-organization (focal contacts, for example, which represent sites of closest juxtaposition to the underlying ECM form within minutes after SGF addition to quiescent cells) (Fig. 1). The genes induced following SGF stimulation encode transcription factors as well as proteins that contribute to the formation or stabilization of serum-induced structural changes. These cytostructural level events are thought to modulate cell-to-substrate adhesion that is required for progression from G_1 through S phase, possibly by influencing the expression of secondary genes including cell cycle regulators and modulators of DNA synthesis. The manner in which SGF stimulation and cellular adhesion interact to influence gene expression and cellular growth control has been the subject of recent studies.

ADHESION-DEPENDENT GROWTH CONTROL

Proliferation of normal adhesion-dependent cells requires both serum and an appropriate supporting matrix; deficiencies in either requirement usually result in growth-arrest [Stoker et al., 1968]. Removal of growth factors (in the form of serum) or culture in suspension (as in agar or methocel) is sufficient to induce entry into a non-cycling or quiescent "growth" condition. Re-activation of quiescent cells by re-introduction of serum is a convenient method to induce synchronous entry into the proliferative cycle. Serum "activation" of previously quiescent cells is a complex process accompanied by an initial rapid and subsequent transient expression of growth-associated transcription factors and specific structural genes. Approximately 30 to 100 such genes are induced with IER kinetics (i.e., rapid transcriptional activation without the requirement for de novo protein synthesis) following serum stimulation. Prominant among the IER genes are those encoding certain transcription factors (e.g., c-*fos*, c-*jun*, c-*myc*), which are thought to regulate the more delayed expression of "late" or secondary genes that encode cyclins, key regulators of cell cycle progression, and "metabolic" genes including modulators of DNA synthesis [Muller et al., 1993]. In addition, stimulation of quiescent fibroblasts into proliferative phase growth is accompanied by the induced expression of proteins comprising the structural link between the ECM and the internal cytoskeleton [Ryseck et al., 1989; Muller et al., 1993]. Certain of these "architectural" elements are also members of the IER gene family. Actin, tropomyosin, alpha-actinin, vinculin, and β1 integrin, for example, are all induced with IER kinetics following serum stimulation of quiescent cells [e.g., Ryseck et al., 1989]. Genes encoding specific elements of the ECM as well as ECM-active proteases are also regulated as a function of cell growth activation [Ryseck et al., 1989; Grimaldi et al., 1986]. This observation suggests a coordination of events designed to modulate cell-to-substrate adhesion during the early stages of growth stimulation. The precise pattern of expression of such genes in the G_0 to G_1 transition period suggests a role for these structural and ECM-degrading proteins in control of cell growth. The G_1 to S phase transition also appears to be influenced by cell-to-sub-

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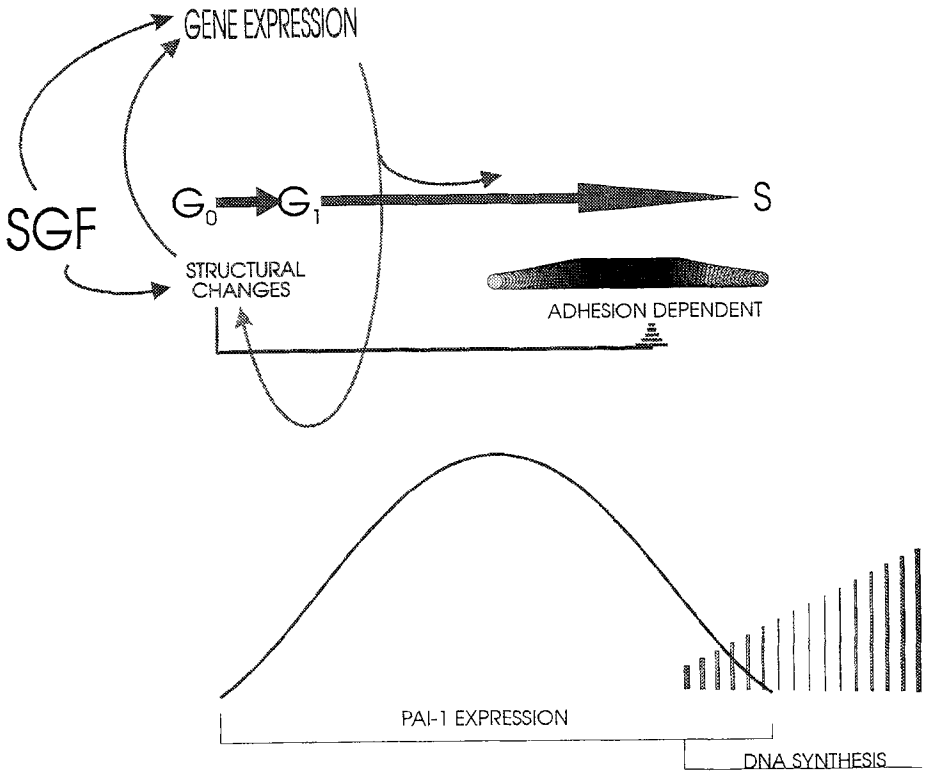

Fig. 1. A model of regulatory events required for serum-induced cell cycle progression.

strate adhesion. Decreased cellular spreading, resulting in reduced adhesion, significantly inhibited DNA synthesis indicating blocked entry into S phase [Folkman and Moscona, 1978]. More recent data has indicated that loss of anchorage precludes G_1 to S phase transit by inhibiting cyclin A mRNA accumulation, a regulator of S phase progression [Guadagno et al., 1993]. Collectively, these data suggest that cell cycle progression from G_0 through S phase likely requires the concerted interaction of growth factor-activated transduction pathways and appropriate (i.e., permissive) restructuring of cell-to-substrate adhesion.

PLASMIN-BASED PERICELLULAR PROTEOLYTIC CASCADE AS A REGULATOR OF CELLULAR ADHESION

In addition to altering gene expression, serum stimulation of adherent quiescent cells results in rapid, often dramatic changes in cytoarchitecture, [Ryan and Higgins, 1994]. Transcytoplasmic actin filaments and their associated focal contact structures form quickly after serum stimulation, suggesting a role in growth control [Ridley and Hall, 1992]. Such contacts function to link the cytoskeleton to the underlying matrix through specific transmembrane receptors (integrins) and may serve to organize a competent signal transducing complex [Burridge et al., 1988]. Since adhesion is necessary for continued cellular proliferation, careful regulation of focal contact structures, which form the closest link of the cell to its underlying matrix [Burridge et al., 1988], would likely impinge on cellular growth control. Regulating contact site formation or half-life can be accomplished by controlled, plasmin-mediated proteolysis (remodeling) of the ECM [Laiho and Keski-Oja, 1989]. The activity and formation of plasmin is carefully regulated by urokinase plasminogen activator (uPA). Focalized localization of active uPA, through the uPA receptor, restricts the locale and activity of plasmin to focal contacts [Pollanen et al., 1987]. uPA function, in turn, is regulated by its fast acting type-1 inhibitor (PAI-1), which is diffusely localized proximal to the focal contact structure proper [Ryan and Higgins, 1994]. PAI-1 appears to stabilize cell-to-substrate adhesion, possibly by maintaining the integrity of the underlying ECM. In so doing, PAI-1 may be able to prolong the life of pre-existing contact sites while creating an environment permissive for development of new adhesive complexes [Ciambrone and McKeown-Longo, 1990]. The balance between expression of both uPA and PAI-1, therefore, may well influence proteolytic activity within the cellular undersurface thereby affecting the stability of the associated contact structures. Events that

modulate expression of uPA and/or PAI-1 would ultimately be expected to affect such basic events as cell-to-substrate adhesion, adhesion-dependent gene regulation, and cell growth control.

PAI-1 and uPA expression appear to be coordinately regulated in a cell cycle-dependent manner. Mitogen stimulation of quiescent cells induces expression of transcripts encoding uPA [Grimaldi et al., 1986] and PAI-1 [Ryan and Higgins, 1994], in each case, with IER kinetics. Subsequent maintenance of PAI-1 expression in mid to late G_1 (4 to 8 h after serum stimulation in growth activated NRK cells), while still transcriptionally regulated, requires protein synthesis which is a metabolic "hallmark" of secondary response expression kinetics (Ryan et al., manuscript submitted). Such IER characteristics following serum stimulation of both uPA and PAI-1 stresses the potential importance of these genes in the early stages of growth activation (i.e., G_0 to G_1 transition). Secondary response kinetics of PAI-1 late in G_1, however, additionally suggest a role for this gene product in regulating the G_1 to S phase transition.

CELLULAR ADHESION AND GENE REGULATION

Under appropriate circumstances, adhesion-dependent signal transduction events regulate both G_0/G_1 and G_1/S progression [Guadagno et al., 1993; Schlaepfer et al., 1995] as well as the expression of growth-related gene products associated with these transition states. Adhesion or integrin occupancy alone under culture conditions that abrogate cell spreading appears sufficient to elicit Na^+/H^+ exchange, influence inositol lipid turnover, and induce the expression of certain IER genes (c-*fos*, c-*myc*, actin), all of which are characteristic of G_0/G_1 transit [reviewed in Schwartz and Ingber, 1994; Dike and Farmer, 1988]. Without signals provided by SGF stimulation, quiescent cells activated by adhesion alone are not capable of progressing through S phase. SGF activation of quiescent 3T3 or NRK cells in the absence of signal transduction events provided by cell-to-substrate adhesion induces the expression of c-*fos*, c-*myc*, and actin indicating G_0 to G_1 transit. These cells do not continue to progress from G_1 through S phase as evidenced by the failure to (1) synthesize DNA, (2) phosphorylate histone H1, and (3) accumulate late G_1/S phase transcripts including cyclin A, thymidine kinase, and histone H4 [Guadagno and Assoian, 1991; Guadagno et al., 1993]. Therefore, entry into the cell cycle can be regulated by either serum or adhesion; progression through S phase, however, requires both serum and adhesion.

The serum and adhesion requirements for entrance into DNA synthetic phase suggests a level of gene regulation that involves a concomitant interaction between at least two distinct signaling events. Indeed, such an interaction has been observed for PAI-1 gene regulation during G_1 transit in synchronized NRK cells (Ryan et al., manuscript submitted). Serum stimulation of quiescent, non-adherent NRK cells induced PAI-1 expression without de novo protein synthesis. Although adhesion, in and of itself, is insufficient to induce PAI-1 synthesis, attachment to tissue culture plastic attenuates the peak level of PAI-1 expression and extends the duration of serum-stimulated PAI-1 induction in a protein synthesis-dependent manner. Serum induced expression of at least two other growth associated genes, c-*myc* and actin, also appears to be modulated by adhesion [Guadagno and Assoian, 1991]. In addition, the expression of other components and regulators of the ECM has previously been shown to be regulated by cell-to-substrate adhesion. Laminin, type IV collagen, and fibronectin expression in mouse mammary epithelial cells is down-regulated following culture on type I collagen [Streuli and Bissell, 1990]. Expression of collagenase and stromelysin, two ECM regulators, in rabbit synovial fibroblasts is induced by signals provided through the fibronectin receptor [Werb et al., 1989]. Fibronectin receptor-generated signals induce collagenase expression through AP-1 and PEA-3 binding sites within the proximal 5′ flanking region (-90 to -67 nt) of the human collagenase gene [Tremble et al., 1995]. The ability of appropriate cell-to-substrate adhesion to regulate cell cycle progression and the expression of components and/or regulators of cellular adhesive complexes and contact sites, therefore, suggests that a universal feedback mechanism, possibly involving AP-1, may be necessary for continued cell cycle progression. Within this model, cellular adhesion may be viewed as influencing the expression of genes whose products function to modulate cell-to-substrate adhesion.

Cellular proliferation and gene reprogramming in response to adhesion are likely mediated by integrins [reviewed by Juliano and Haskill, 1993]. Two hypotheses, that are not necessarily mutually exclusive, have been put forward to explain the role of integrins in the growth response. The first suggests that integrin mediated signalling events require a particular cell shape and cytoarchitecture that the integrins themselves organize. Clustered, ligand-bound integrin molecules within focal contacts do, indeed, interact with internal cytoskeletal components [Miyamoto et al., 1995] implicating integrin-mediated ECM binding in cell shape control and cytoskeletal organization. Moreover, particular cytoarchitectural constraints may influence certain biosynthetic pathways. Compared to their exponentially growing counterparts, for example, quiescent cells display reduced macromolecular (hnRNA, mRNA, rRNA, protein, DNA) synthesis [Darzynkiewicz et al., 1980; Be-

necke et al., 1978]. Addition of high concentrations of serum or attachment of suspended cells onto tissue culture plastic results in an increase in cellular RNA and protein content prior to re-entry into DNA synthetic phase [Darzynkiewicz et al., 1980; Benecke et al., 1978]. An alternative hypothesis suggests that growth activation and the associated reprogramming of gene expression involve integrin-mediated signalling pathways. Clustering integrin molecules without necessarily occupying the ligand binding domain has been shown, in fact, to enhance tyrosine phosphorylation of a tyrosine kinase found within the focal contact ($pp125^{fak}$) [reviewed by Juliano and Haskill, 1993; Miyamoto et al., 1995]. Activated $pp125^{fak}$ binds the SH2 domains of $pp60^{src}$, a non-receptor tyrosine kinase [Cobb et al., 1994] and GRB2 [Schlaepfer et al., 1995], an SH2/SH3 adaptor protein that links growth factor receptor tyrosine kinase signal transduction to the Ras/MAPK pathway through the Ras GDP/GTP exchange protein Sos. Indeed, $pp125^{fak}$ stimulation as a consequence of ECM-integrin interaction does coincide with MAPK phosphorylation [Schlaepfer et al., 1995]. Activation of an integrin-mediated signalling pathway, within the context of an appropriate cytoarchitecture, may likely influence if not initiate adhesion-mediated reprogramming of gene expression and the subsequent effect on cellular growth control.

SUMMARY

Appropriate cell-to-substrate adhesion together with SGF stimulation is necessary to initiate and continue cell cycle progression of growth arrested cells. Adhesion-dependent signaling events, which likely occur through integrin receptors specifically organized with cytoskeletal components within focal contacts, can induce expression of specific genes and stimulate quiescent cells into the growth cycle. The mechanisms as to how: (1) cell-to-substrate adhesion complexes are formed and maintained, (2) adhesion-dependent signal transduction events interface with SGF initiated signalling events, (3) adhesion influences expression of growth-state regulated genes, and (4) an appropriate cytoarchitectural environment may coordinate these events to regulate cellular growth are unclear. While it is apparent that defining these mechanisms would be critical to understanding the basic events which control cell growth, many of the mechanisms are just beginning to be addressed and understood.

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