Journal of Cell Science 109, 1165-1172 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JCS4196

COMMENTARY

Signal transduction by the $\alpha_6\beta_4$ integrin: charting the path between laminin binding and nuclear events

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INTRODUCTION

Basement membranes contribute to the organization of tissues and organs and play dynamic roles in tissue morphogenesis, wound repair and cellular trafficking (Paulsson, 1992; Timpl, 1989). The biological effects of basement membranes can in large part be attributed to laminins, a large family of homologous extracellular matrix proteins expressed in a tissue specific fashion (Engvall, 1993). By binding to cell surface integrins, laminins promote cell adhesion and exert profound influences on cell survival, proliferation and differentiation (Mercurio, 1995). It is likely that the effects of laminins on cellular behavior depend on the ability of integrins to participate in intracellular signaling (Clark and Brugge, 1995; Giancotti and Mainiero, 1994; Schwartz et al., 1995). However, the mechanisms by which integrins transduce signals accross the plasma membrane to ultimately affect gene expression are incompletely understood. In this commentary I will focus on the signaling functions of the laminin-binding integrin $\alpha_6\beta_4$. Recent studies have revealed that $\alpha_6\beta_4$ is associated with a cytoplasmic tyrosine kinase and linked to the *ras* signaling pathway by a series of SH2- and SH3-containing adaptor molecules (Mainiero et al., 1995). These results provide a molecular basis to the previously recognized ability of basement membranes to affect gene expression and may serve as a paradigm in future studies focusing on the signal transduction mechanisms of other integrins.

ROLE OF $\alpha_6\beta_4$ IN MORPHOGENESIS AND DEVELOPMENT

The $\alpha_6\beta_4$ integrin is a receptor for various laminin isoforms (Lee et al., 1992; Niessen et al., 1994; Spinardi et al., 1995) and it binds with the highest apparent affinity to laminin 5 and laminin 4 (Spinardi et al., 1995). In accordance with its ligand binding specificity, $\alpha_6\beta_4$ is expressed in cell types which interact with laminin-rich matrices in vivo, most notably epithelial (Kajiji et al., 1989), endothelial (Kennel et al., 1992; Klein et al., 1993) and Schwann cells (Sonnenberg et al., 1990; Einheber, 1993). The expression of $\alpha_6\beta_4$ and its laminin ligands is regulated during embryonic development and differentiation suggesting that $\alpha_6\beta_4$ may have important functions during morphogenesis.

In stratified epithelia, such as the epidermis, the expression of $\alpha_6\beta_4$ is restricted to those keratinocytes which abut the basement membrane and are endowed with proliferative capacity (Kajiji et al., 1989). Keratinocytes withdraw from the cell cycle and begin to differentiate as soon as they leave the basement membrane to migrate to the upper layers of skin (Hall and Watt, 1989) and this process can be replicated in vitro by detaching cultured keratinocytes from their extracellular matrix (Green, 1977) which is particularly rich in laminin 5 (Carter et al., 1991; Rousselle et al., 1991). In addition, squamous carcinoma cells endowed with high proliferative potential often express elevated levels of $\alpha_6\beta_4$ (Kimmel and Carey, 1986; Wolf et al., 1990). The correlation between expression of $\alpha_6\beta_4$ and cell proliferation revealed by these observations suggests that this integrin may provide epithelial cells with a signal important for cell cycle progression.

The $\alpha_6\beta_4$ integrin is abruptly downregulated at the onset of keratinocyte differentiation. This is in striking contrast with the gradual disappearance of other laminin binding integrins during epidermal differentiation (Carter et al., 1990a,b). It is likely that a post-transductional mechanism contributes to the rapid disappearance of $\alpha_6\beta_4$ from differentiating keratinocytes. The cytoplasmic domain of β_4 is cleaved in vitro and in cultured cells by the calcium-dependent protease calpain and there is evidence suggesting that this cleavage occurs also in vivo in the basal layer of epidermis (Giancotti et al., 1992). Notably, calpain digestion results in the release of a portion of the β_4 tail that is predicted to be essential for linkage to the hemidesmosomal cytoskeleton (Spinardi et al., 1993). Since the cytoskeletal interactions mediated by the cytoplasmic domain of β_4 appear to be important for long-term stable adhesion to the basement membrane (Spinardi et al., 1995), it is possible that cleavage of the β_4 tail facilitates detachment from the basement membrane at the onset of differentiation. In addition to regulating calpain activity, calcium is a trigger of epidermal differentiation (Hennings et al., 1980) and its concentration in the cytosol of epidermal cells rises as they differentiate (Menon et al., 1985). The ability of calcium to simultaneously control $\alpha_6\beta_4$-mediated adhesion and differentiation

Key words: $\alpha_6\beta_4$ integrin, Hemidesmosome, Signaling

may thus serve to couple the reversal of adhesion to the onset of differentiation in epidermal cells. Alternatively, the downregulation of integrin-mediated adhesion to the basement membrane may in itself represent a signal contributing to differentiation, as it is suggested by the observation that detachment per se is a sufficient stimulus for epidermal differentiation (Green, 1977).

Immunohistochemical studies have shown that the $\alpha_6\beta_4$ integrin is expressed in a subset of small vessels (Kennel et al., 1992). This observation may reflect an involvement of $\alpha_6\beta_4$ in angiogenesis. Recent studies on the expression of various integrins during dermal angiogenesis in vivo have indicated that $\alpha_6\beta_4$ is enriched at the tip of endothelial sprouts (Enenstein and Kramer, 1994), where it colocalizes with laminins (Jerdan et al., 1991). Furthermore, the prototypic angiogenic factor FGF-2 upregulates the expression of $\alpha_6\beta_4$ in capillary endothelial cells cultured in vitro (Klein et al., 1993). Thus, it is possible that ligation of $\alpha_6\beta_4$ at the growing tip of new vessels provides the endothelial cells with a growth stimulatory signal.

Ensheathment and myelination of axons by Schwann cells in the peripheral nervous system is a process that requires contact with the basement membrane (Bunge et al., 1986; Carey et al., 1986). The $\alpha_6\beta_4$ integrin may be also involved in this process. When undifferentiated, proliferating Schwann cells are co-cultured with neurons under conditions which promote withdrawal from the cell cycle and myelination, they are induced to express de novo significantly high levels of $\alpha_6\beta_4$ (Einheber et al., 1993). In accordance with this observation, β_4 levels also increase during myelination of developing peripheral nerves in vivo (Feltri et al., 1994). The basement membrane that the Schwann cells deposit at the onset of myelination contains the $\alpha_6\beta_4$ ligands laminin 2 and 4 (Sanes et al., 1990; Marinkovich et al., 1992), hence it is likely that $\alpha_6\beta_4$ interacts with these extracellular matrix proteins during myelination. Recent studies are consistent with the hypothesis that the interaction of differentiating Schwann cells with laminins is physiologically important. In particular, the observation that the *dy*/*dy* mouse, which carries a mutation in the α_2 subunit (LAMA2) gene and therefore lacks both laminin 2 and 4 (Sunada et al., 1994; Xu et al., 1994), develops a form of muscular dystrophy accompanied by peripheral nerve degeneration suggests that $\alpha_6\beta_4$ provides the Schwann cells with a signal important for survival and/or differentiation.

Finally, $\alpha_6\beta_4$ is expressed transiently during T-lymphocyte development. $CD4^-/CD8^-$ pre-T lymphocytes begin to express $\alpha_6\beta_4$ when they enter the thymus and lose the integrin soon after when they become single positive cells (Wadsworth et al., 1992). The thymus contains laminin 2 and 5 (Chang et al., 1993; Jaspars et al., 1993). Thus, it is conceivable that laminin binding to $\alpha_6\beta_4$ participates in regulating the homing or differentiation of pre-T cells. In accordance with this hypothesis, it has been recently reported that T lymphocyte development is defective in the laminin-deficient *dy/dy* mouse (Magner et al., 1995). These observations suggest that $\alpha_6\beta_4$ may be involved in T-cell differentiation.

CHANGES DURING TUMOR PROGRESSION

Neoplastic transformation can profoundly affect the level of expression of the $\alpha_6\beta_4$ integrin, but the direction of the change seems to depend on tumor type. Several studies indicate that $\alpha_6\beta_4$ is expressed at high levels in squamous carcinomas of lung, skin, oral cavity and cervix (Carico et al., 1993; Kimmel and Carey, 1986; Mariani-Costantini et al., 1990; Wolf et al., 1990). In fact, the β_4 subunit was known before its time as a cell surface molecule specifically expressed in squamous carcinomas (Kimmel and Carey, 1986). Although it is not clear if $\alpha_6\beta_4$ is genuinely upregulated in individual squamous carcinoma cells as compared with normal basal keratinocytes, it is evident that the expression of the integrin in squamous cancers is no longer restricted to the basal cell layer, but it extends to several suprabasal layers (Carico et al., 1993; Kimmel and Carey, 1986; Savoia et al., 1993). In addition, $\alpha_6\beta_4$ is diffusely distributed over the entire surface of squamous carcinoma cells (Kimmel and Carey, 1986; Savoia et al., 1993), perhaps because of a defective interaction with the hemidesmosomal cytoskeleton (Shenk, 1979; Bergstraesser et al., 1995). The suprabasal expression of the integrin is likely to reflect an expansion of the stem cell compartment in these malignancies. If this is so, the areas of focal loss of $\alpha_6\beta_4$ detected in some squamous tumors (Jones et al., 1993) may comprise cells which have at least partially differentiated and therefore lost expression of the integrin. To further investigate this hypothesis, it will be important to determine if the $\alpha_6\beta_4$-negative tumor cells are less malignant than those expressing normal or increased levels of the integrin.

Several observations support the notion that the $\alpha_6\beta_4$ integrin is a positive regulator of epidermal cell malignancy. Studies in a well characterized mouse skin carcinogenesis model have indicated that the levels of $\alpha_6\beta_4$ steadily increase during tumor progression (Tennenbaum et al., 1992). In particular, it is significant that $\alpha_6\beta_4$ levels increase only in those skin papillomas which will become carcinomas and that this occurs prior to their full conversion to malignancy and independently of whether the tumors were induced by retroviral transduction or chemical carcinogens (Tennenbaum et al., 1993). In addition, while in normal skin and benign papillomas the β_4 subunit is associated exclusively with the canonical form of α_6, carcinoma cells also express the α_{6B} splicing variant subunit which may enable them to interact more avidly with laminins (Tennenbaum et al., 1995). Finally, it may not be coincidental that carcinoma cells invading the adjacent stroma express on their surface significant amounts of the $\alpha_6\beta_4$ ligand laminin 5 (Pyke et al., 1994, 1995) and carcinoma cells selected for their ability to invade a laminin-rich extracellular matrix display high levels of $\alpha_6\beta_4$ (Dedhar et al., 1993). These observations suggest that increased levels of $\alpha_6\beta_4$ are selected for during tumor progression because they may facilitate the invasive growth of neoplastic epidermal cells.

In apparent contrast with these findings, several observations indicate that $\alpha_6\beta_4$ is downregulated in adenocarcinomas of breast and prostate (Knox et al., 1994; Koukoulis et al., 1991; Natali et al., 1992). It is possible that, while in normal epidermal cells $\alpha_6\beta_4$ functions as a positive regulator of cell growth, in normal breast and prostate cells it contributes to cell cycle withdrawal and differentiation. If this is the case, successful tumor growth would be facilitated by an upregulation of $\alpha_6\beta_4$ in those cell types in which the integrin stimulates cell

growth and by its downregulation in those others in which the integrin promotes differentiation.

STRUCTURE AND CYTOSKELETAL INTERACTIONS

The $\alpha_6\beta_4$ integrin is among integrins unique in structure and subcellular localization. Although the extracellular portion of $\alpha_6\beta_4$ is homologous to that of other known integrins, the cytoplasmic domain of β_4 subunit is over 1,000 amino acids long and bears no homology with the short cytoplasmic domains of other known β subunits (Hogervorst et al., 1990; Suzuki and Naitoh, 1990). It contains towards its C terminus two pairs of type III fibronectin (Fn)-like modules separated by a 142 amino acid sequence (Connecting Segment) which appears to be the target of multiple regulatory mechanisms, including tyrosine phosphorylation (Mainiero et al., 1995) and proteolytic processing (Giancotti et al., 1992). In contrast to β_1 and β_3 integrins which localize to focal adhesions, the $\alpha_6\beta_4$ integrin is found concentrated in hemidesmosomes (Carter et al., 1990a,b; Stepp et al., 1990). This observation indicates that $\alpha_6\beta_4$ interacts with the keratin filament system, and not with the actin cytoskeleton.

The hemidesmosomes are punctuate junctions connecting the basal cells of stratified and complex epithelia to the basement membrane (Schwarz et al., 1990; Legan et al., 1992). At the ultrastructural level they appear as tripartite structures with an electron dense juxta-membranous plaque and an innermost plaque linked to the keratin filaments (Shienvold and Kelly, 1976). The juxta-membranous plaque is connected to the innermost plaque by short filaments. Furthermore, short threads of extracellular matrix called anchoring filaments connect the extracellular aspect of hemidesmosomes to the lamina lucida (Ellison and Garrod, 1984).

The hemidesmosomes are complex structures. Despite the functional importance of hemidesmosomes, relatively little is known about the structure and function of many of their components. Fig. 1 shows a schematic model of the structure and molecular composition of hemidesmosomes. The anchoring filaments contain laminin 5 and the binding of this matrix protein to $\alpha_6\beta_4$ may be the primary determinant in the establishment and maintainance of hemidesmosomes. However, in addition to $\alpha_6\beta_4$, the hemidesmosomes contain another transmembrane protein potentially involved in cell adhesion, the Bullous Pemphigoid Antigen 2 (BPAG 2) (Giudice et al., 1992). Recent results suggest that BPAG2 may interact with the α_6 subunit and perhaps cooperate with $\alpha_6\beta_4$ in mediating cell adhesion to the basement membrane (Hopkinson et al., 1995). All the other known elements of hemidesmosomes are intracellular and appear to reside in the inner plaque. They include the Bullous Pemphigoid Antigen 1 (BPAG1), which is the major target of the autoantibodies present in the blistering skin disease Bullous Pemphigoid (Sawamura et al., 1991; Tanaka et al., 1991), the HD-1 protein (Hieda et al., 1992), and the 6A5 antigen (Kurpakus et al., 1991). BPAG1 contains a region of homology with the desmosomal protein desmoplakin (Sawamura et al., 1991). Since the homology falls within a desmoplakin domain that directly binds to keratins (Stappenbeck and Green, 1992; Kouklis et al., 1994), it is likely that BPAG1 also interacts with the keratin filaments, thereby linking the innermost plaque of hemidesmomes to the cytoskeleton. In accordance with this hypothesis, the hemidesmosomes of BPAG1 knock-out mice lack the inner plate and are not connected to the keratin cytoskeleton (Guo et al., 1995). The molecular interactions between the transmembrane and intracellular components of hemidesmosomes are incompletely understood because the composition of the short filaments connecting the inner plaque to the juxta-membranous plaque is not known.

The expression of various deletion mutant forms of β_4 in hemidesmosome forming cells has revealed that the association of $\alpha_6\beta_4$ with the hemidesmosomal cytoskeleton is mediated by the β_4 cytoplasmic domain and, specifically, by a 303 amino acid region containing the first pair of type III Fn-like repeats and the Connecting Segment (Spinardi et al., 1993). The observation that the extracellular domain of $\alpha_6\beta_4$ binds to laminin 5 and the specialized cytoplasmic tail of β_4 subunit interacts with the hemidesmosomal cytoskeleton suggests that this integrin plays a crucial role in the assembly of hemidesmosomes and their linkage to the keratin filament system. In accordance with this hypothesis, the introduction of a truncated tail-less β_4 subunit in cells possessing endogenous $\alpha_6\beta_4$ integrins and hemidesmosomes results in a dominant negative effect on hemidesmosome assembly (Spinardi et al., 1995). Since the tail-less integrin retains the ability to bind laminins, its dominant negative effect is likely to result from its ability to co-cluster with the endogenous wild-type receptor and block a conformational change or a signal necessary for hemidesmosome assembly. Interestingly, the cells which express the tail-less integrin do not show a defective interaction with laminins in short term adhesion assays, but they are significantly rounder and more detached from the culture substratum than control cells. This observation suggests that the main function of hemidesmosomes is to stabilize the $\alpha_6\beta_4$-mediated adhesion to the basement membrane.

Several observations indicate that the transmembrane connection between the basement membrane and the keratin filament system mediated by $\alpha_6\beta_4$ plays a crucial role in maintaining the integrity of skin and other stratified epithelia. Mutations in the β_3 and γ_2 laminin subunit genes (Aberdam et al., 1994; Pulkkinen et al., 1994a,b) and lack of expression of laminin 5 (Domloge-Hultsch et al., 1992) have been detected in the lethal form (Herlitz type) of Junctional Epidermolysis Bullosa (JEB), a blistering skin disease in which the hemidesmosomes are defective and the epidermis detaches from the basement membrane. Furthermore, mutations in the β_4 gene and greatly reduced or often absent expression of $\alpha_6\beta_4$ have been observed in a subset of JEB which is accompanied by pyloric atresia (Gil et al., 1994;Vidal et al., 1995). Finally, mice in which the β_4 gene has been knocked out display extensive detachment of the skin and other epithelial abnormalities (Arnoud Sonnenberg, personal communication). Since the β_4 knock-out mice die soon after birth, to examine the potential involvement of $\alpha_6\beta_4$ in Schwann cell myelination and T-cell differentiation it will be necessary to generate chimeric mice or to rescue the mutant mice from blistering by targeting the expression of a transgenic wild-type β_4 subunit to the skin. Although the above mentioned studies may have only partially uncovered the developmental roles of $\alpha_6\beta_4$, they have provided clear indication that the interaction of $\alpha_6\beta_4$ with laminin 5 at hemidesmosomes is of crucial physiological importance.

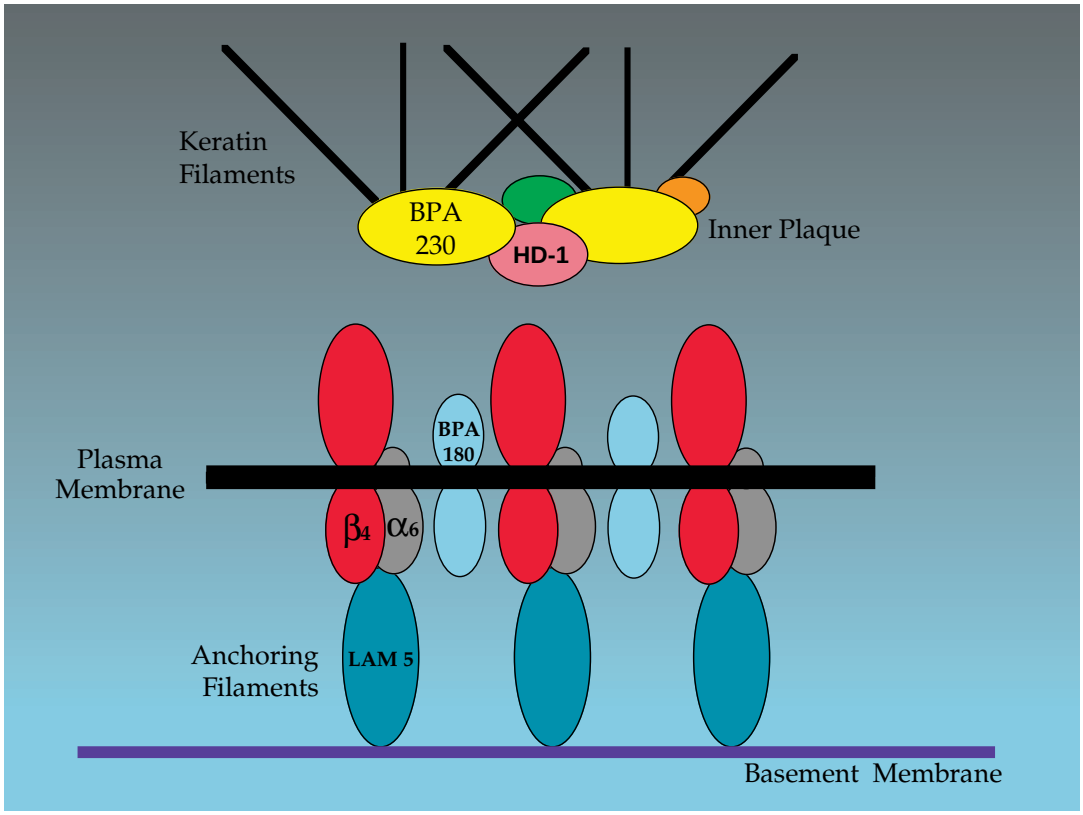

Fig. 1. Molecular architecture of the hemidesmosome.

INTRACELLULAR SIGNALING

The multifarious effects of laminins on cellular behavior, in particular their ability to influence proliferation and differentiation, suggest that laminin-binding results in intracellular signaling. What are the mechanisms by which the $\alpha_6\beta_4$ integrin transduces biochemical signals to the cell interior? The results of a recent series of experiments suggests that $\alpha_6\beta_4$ relies on its association with an intracellular tyrosine kinase for signal transduction (Mainiero et al., 1995). In this respect $\alpha_6\beta_4$ can be considered a binary tyrosine kinase receptor similar to many cytokine and immune receptors, which do not possess an intracellular catalytic domain but are physically and functionally associated with cytoplasmic tyrosine kinases (Kishimoto et al., 1994; Weiss and Littman, 1994). Laminin binding to $\alpha_6\beta_4$ causes activation of the associated kinase and consequently tyrosine phosphorylation of the β_4 subunit cytoplasmic domain. Since these events can be replicated by exposing the cells to beads coated with anti-$\alpha_6\beta_4$ antibodies, the mechanism by which ligand binding causes tyrosine phosphorylation of β_4 is likely to include dimerization or oligomerization of the integrin on the plasma membrane. This event is likely to bring the integrin-associated kinase in close proximity to its target sequences in the β_4 tail and to induce *trans* phosphorylation and activation of the kinase.

Immunoprecipitation experiments have indicated that the adaptor protein Shc forms a tight complex with the tyrosine phosphorylated β_4 subunit and is thereby phosphorylated on tyrosine residues, presumably by the integrin-associated kinase. The subsequent recruitment to the plasma membrane of the other adaptor protein Grb2, which exists in a complex with the *ras* GTP exchange factor mSOS, potentially links $\alpha_6\beta_4$ to the *ras* signaling pathways. Indeed, *ras* GTP-loading experiments have indicated that ligation of $\alpha_6\beta_4$ results in a significant activation of *ras* (F. Mainiero and F. G. Giancotti, unpublished results). The linkage of $\alpha_6\beta_4$ to *ras* and the various MAP kinase pathways directly or indirectly regulated by *ras* provides a mechanism by which laminins could regulate gene expression. Moreover, since the MAP kinase pathway activated by *ras* has been implicated in controlling proliferation or differentiation depending on the cellular context (Cowley et al., 1994), the signal transduction mechanism described above may explain the apparently paradoxical effects of laminins on morphogenesis and tumor progression.

In apparent contrast with the above results, it has been recently reported that overexpression of wild-type, but not tailless β_4 induces $p21^{WAF1/Cip1}$ expression and cell-cycle withdrawal in rectal carcinoma cells (Clarke et al., 1995). However, since this effect appears to occur independently of ligand binding to the integrin, it is possible that the unoccupied $\alpha_6\beta_4$ is somehow linked to the $p21^{WAF1/Cip1}$ pathway of growth suppression and that laminin binding reverses this connection and couples the integrin through Shc and Grb2 to *ras*. The observation that reversion of $\alpha_6\beta_4$-mediated adhesion causes dephosphorylation of an as yet unidentified 80 kDa protein (Xia et al., 1996) is also consistent with the hypothesis that ligated and unligated $\alpha_6\beta_4$ integrins impinge on distinct intracellular pathways.

As discussed above, the assembly of hemidesmosomes is likely to be triggered by the binding of laminin 5 to $\alpha_6\beta_4$ and to require the subsequent interaction of the β_4 cytoplasmic domain with cytoskeletal elements of hemidesmosomes. There is strong evidence indicating that $\alpha_6\beta_4$ signaling is an integral component of this process. The multiple β_4 sites phos-

phorylated in vivo by the $\alpha_6\beta_4$-associated kinase include a tyrosine activation motif (TAM) located in the Connecting Segment. The TAM is a bidentate phosphorylation motif which consists of two closely spaced tyrosine residues followed by a leucine at position + 3 and was originally identified in several immune receptors (Reth, 1989). Interestingly, $\alpha_6\beta_4$ is the first molecule outside the immune system with such a motif. Phenylalanine substitutions at the β_4 TAM disrupt the association of $\alpha_6\beta_4$ with hemidesmosomes, but do not affect recruitment of Shc and Grb2 (Mainiero et al., 1995). This result indicates that phosphorylation of the β_4 TAM is necessary for the assembly of hemidesmosomes, but not for the recruitment of Shc and Grb2. In accordance with the role of tyrosine phosphorylation in hemidesmosome assembly, inhibition of β_4 phosphorylation by the tyrosine kinase inhibitor herbimycin correlates with inhibition of hemidesmosomes (A. Pepe and F. G. Giancotti, unpublished results). Furthermore, the ability of tail-less β_4 to suppress hemidesmosome formation is reminiscent of the dominant negative effect that tail-less tyrosine kinase receptors exert on signal transduction and is consistent with the proposed role of $\alpha_6\beta_4$ signaling in hemidesmosome assembly (Spinardi et al., 1995).

The immune receptor TAMs provide a template for the binding of tyrosine kinases containing two tandem SH2 domains, such as *Syk* and ZAP70. Recruitment of these kinases to the TAMs is coupled with their activation and is crucial for subsequent downstream signaling events from immune receptors (Weiss and Littman, 1994). These observations suggest that the mechanism by which phosphorylation of the β_4 TAM regulates cytoskeletal assembly may involve binding of a protein containing two tandem SH2 domains. It is unlikely that this element is a cytoskeletal element which binds stably to $\alpha_6\beta_4$ because tyrosine phosphorylation of β_4 occurs only transiently after ligand binding. In fact, in stably adherent cells β_4 appears to be completely dephosphorylated, yet most of $\alpha_6\beta_4$ is in hemidesmosomes. In addition, phosphorylation of the TAM is unlikely to be sufficient for the association of $\alpha_6\beta_4$ with the hemidesmosomal cytoskeleton because deletion mutagenesis experiments have indicated that the two type III fibronectin-like modules upstream of the TAM are also required for this process (L. Spinardi and F. G. Giancotti, unpublished results). The analogy with the immune receptors suggests that the β_4 TAM mediates a signal important for the assembly of hemidesmosomes by recruiting a protein kinase, phosphatase or adapter. The tyrosine kinases ZAP70 and *syk* appear to be restricted to the immune system and therefore their tissue distribution does not overlap with that of $\alpha_6\beta_4$. In addition, since the spacing between tyrosine 1422 and 1440 in β_4 is larger than the distance between the tyrosines in immune receptor TAMs, it is likely that the β_4 TAM has a distinct binding specificity and mediates the activation of a novel signal transduction pathway.

The model proposed above is illustrated in Fig. 2. Its major prediction is that the association of $\alpha_6\beta_4$ with the hemidesmososmal cytoskeleton and its linkage to *ras* are mediated by distinct divergent pathways. This suggests the possibility that the two pathways are differentially regulated in vivo, perhaps in response to different laminins or different oligomerization states of the same laminin matrix. Activation of each of the two pathways may also depend on the availability of the signaling components involved and therefore vary

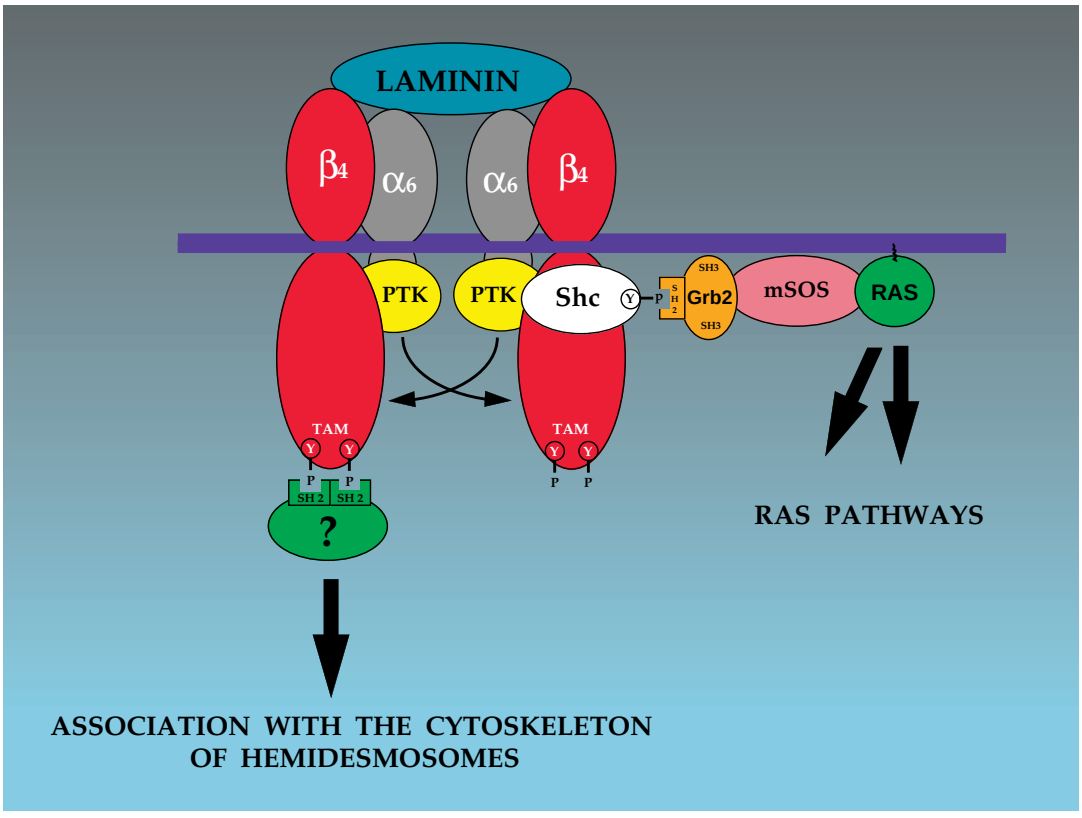

Fig. 2. Model of $\alpha_6\beta_4$-mediated signal transduction.

with cell type and developmental stage. These mechanisms would clearly provide flexibility to cellular responses to laminins.

CONCLUSION AND PERSPECTIVES

The above results delineate the basic mechanism by which the $\alpha_6\beta_4$ integrin transduces biochemical signals accross the plasma membrane and offer a number of avenues for future investigation. At the molecular level, it will be important to identify the tyrosine kinase associated with $\alpha_6\beta_4$ and define all the intracellular pathways activated by the integrin and their target genes. It is likely that $\alpha_6\beta_4$ activates the various MAP kinase pathways controlled by *ras*, but the extent to which each one is stimulated remains to be determined. In addition, the recruitment of Shc and Grb2 may link $\alpha_6\beta_4$ not only to *ras*, but also to other adaptor and effector molecules. A comprehensive understanding of the pathways activated by $\alpha_6\beta_4$ will be a first necessary step to elucidate the molecular basis by which the integrin can influence proliferation and differentiation.

Progression through the cell cycle and differentiation are usually mutually exclusive and require the activation of largely distinct sets of genes. It is intriguing that most growth and differentiation factors activate the *ras*-MAP kinase pathway and that MAP kinase can induce proliferation or differentiation depending on the cell type (Cowley et al., 1994). This observation suggests that MAP kinase may impinge on different transcription factors in different cell types or physiological situations. If this is the case, the final output of signaling through MAP kinase may depend on which set of target factors is available in any given situation. Alternatively, it is possible that the choice between growth and differentiation is controlled by specific pathways distinct from those activated by *ras*. Clearly, it will be important to resolve these issues with regard to signaling from both classical growth factor receptors and integrins.

The relationship between growth factor-dependent signaling and cell adhesion mediated by $\alpha_6\beta_4$ or other integrins needs further investigation. One cannot discount the exciting possibility that the final output of signaling from integrins may depend on the existence of as yet unidentified pathways impinging on genes distinct from those activated by growth and differentiation factors. Alternatively, as the above described studies on $\alpha_6\beta_4$ suggest, the pathways activated by soluble factors and extracellular matrix proteins may be largely overlapping. In both cases, it will be important to understand how the signals are integrated and what determines the quality of the final output.

Experiments of mutagenesis and dominant negative inhibition in cultured cells may help to understand the influence that signaling by $\alpha_6\beta_4$ may have on survival and proliferation. It is likely that the potential role of $\alpha_6\beta_4$ signaling in differentiation will be best addressed by molecular genetics studies in the mouse, especially if it will be possible to separate the adhesive and signaling function of the integrin. Finally, the pathway which controls the assembly of hemidesmosomes is likely to be specifically activated by $\alpha_6\beta_4$. To understand this pathway it will be necessary to identify the signaling component interacting with the phosphorylated β_4 TAM as well as its downtream targets. One possibility is that these targets include one or more cytoskeletal elements of hemidesmosomes. Proper modification of these molecules by the β_4 TAM signaling pathway may be necessary for their subsequent interaction with the β_4 cytoplasmic domain. Clearly, although studying $\alpha_6\beta_4$ has been particularly rewarding, much remains to be learned.

The author is the recipient of awards from the Lucille P. Markey Charitable Trust and the Irma T. Hirschl Trust. Work in his laboratory is supported by grants from the NIH and the USAMRMC.

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