Tyrosine Phosphorylation of Paxillin and pp125^{FAK} Accompanies Cell Adhesion to Extracellular Matrix: A Role in Cytoskeletal Assembly

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Abstract. Cells in culture reveal high levels of protein tyrosine phosphorylation in their focal adhesions, the regions where cells adhere to the underlying substratum. We have examined the tyrosine phosphorylation of proteins in response to plating cells on extracellular matrix substrata. Rat embryo fibroblasts, mouse Balb/c 3T3, and NIH 3T3 cells plated on fibronectin-coated surfaces revealed elevated phosphotyrosine levels in a cluster of proteins between 115 and 130 kD. This increase in tyrosine phosphorylation was also seen when rat embryo fibroblasts were plated on laminin or vitronectin, but not on polylysine or on uncoated plastic. Integrin mediation of this effect was suggested by finding the same pattern of elevated tyrosine phosphorylation in cells plated on the cell-binding fragment of fibronectin and in cells plated on a synthetic polymer containing multiple RGD sequences. We have identified one of the proteins of the 115–130-kD cluster as pp125^{FAK}, a tyrosine kinase recently localized in focal adhesions (Schaller, M. D., C. A. Borgman, B. S. Cobb, R. R. Vines, A. B. Reynolds, and J. T. Parsons. 1992. *Proc. Natl. Acad. Sci. USA.* 89:5192). A second protein that becomes tyrosine phosphorylated in response to extracellular matrix adhesion is identified as paxillin, a 70-kD protein previously localized to focal adhesions. Treatment of cells with the tyrosine kinase inhibitor herbimycin A diminished the adhesion-induced tyrosine phosphorylation of these proteins and inhibited the formation of focal adhesions and stress fibers. These results suggest a role for integrin-mediated tyrosine phosphorylation in the organization of the cytoskeleton as cells adhere to the extracellular matrix.

THE extracellular matrix (ECM)[1] affects many aspects of cell behavior, including the migratory properties of cells, their morphology, growth characteristics, and differentiation (Hay, 1981; Hynes, 1990). The means by which the ECM exerts these effects are largely unknown, but there is evidence that many of these actions are mediated through integrins, the family of ECM receptors found on most cells. Integrins are $\alpha\beta$ heterodimers; each subunit has a large extracellular domain, spans the membrane once, and has a short cytoplasmic sequence (Buck and Horwitz, 1987; Hynes, 1987; Ruoslahti and Pierschbacher, 1987). Multiple α and β subunits have been identified (Albelda and Buck, 1990; Hemler, 1990; Hynes, 1992). Some subunits are shared by many integrins (e.g., β_1), whereas others appear to be unique. During the last few years much has been learned about the interaction of integrins with particular ECM components such as fibronectin or vitronectin. In many cells in culture, integrins are clustered at sites of adhesion to the underlying ECM in regions known as focal adhesions (focal contacts or adhesion plaques) (for review, see Burridge et al., 1988). The extracellular face of focal adhesions provides attachment to matrix components adsorbed onto the plastic or glass culture surface, whereas the cytoplasmic face provides a site of attachment for bundles of actin filaments (stress fibers). Evidence from in vitro binding experiments indicates that integrins can interact with at least two cytoskeletal proteins, talin (Horwitz et al., 1986) and α-actinin (Otey et al., 1990). Along with several other cytoskeletal proteins, talin and α-actinin are concentrated at the cytoplasmic face of focal adhesions (Burridge et al., 1988).

Focal adhesions are thought to be important not only as structural links between the ECM and the cytoskeleton, but also as sites of signal transduction from the ECM. Several potential regulatory enzymes have been identified in focal adhesions (Burridge et al., 1988). These include protein kinase C (Jaken et al., 1989) and several tyrosine kinases (Rohrschneider, 1980; Rohrschneider and Gentry, 1984; Rohrschneider and Najita, 1984). Considerable attention has been directed toward the tyrosine kinase pp60$^{v\text{-}src}$, which is localized to the residual focal adhesions of cells transformed by Rous sarcoma virus (Rohrschneider, 1980). Pp60$^{v\text{-}src}$ has not yet been detected in the focal adhesions of normal cells, but elevated phosphotyrosine has been noted

1. *Abbreviations used in this paper*: ECM, extracellular matrix; REF, rat embryo fibroblasts.