JCB Comment

FAK and paxillin: regulators of N-cadherin adhesion and inhibitors of cell migration?

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FAK and paxillin are important components in integrin-regulated signaling. New evidence suggests that these two proteins function in crosstalk between cell–matrix and cell–cell adhesions. Further, new insight suggests that under some conditions these proteins inhibit cell migration, in contrast to their established roles in several cell systems as positive regulators of cell adhesion and migration.

FAK and paxillin are two focal adhesion–associated proteins that function in transmitting signals downstream of integrins. These signals control important biological events, including cell migration, proliferation, and survival. Yano et al. (2004) now report intriguing findings that may stimulate reevaluation of the role of FAK and paxillin in the regulation of cell motility. Using a small interfering RNA (siRNA) strategy, FAK and paxillin signaling was impaired, resulting in increased cell migration and suggesting these proteins normally function to retard motility. This observation will prove controversial, as many reports have implicated FAK as a positive regulator of cell motility, and several reports have demonstrated a similar function for paxillin. In addition, siRNA-mediated knockdown of FAK and paxillin resulted in impaired assembly of N-cadherin–containing cell–cell adhesions, suggesting a novel role for these proteins in the crosstalk between cell–matrix and cell–cell adhesions.

Given the plethora of evidence implicating FAK as a positive regulator of cell migration, how can the discrepant results presented by Yano et al. (2004) be resolved with the literature? The contradictory results are not simply explained by cell type differences, as inhibition of FAK and paxillin by siRNA impairs motility in both HeLa cells (of epithelial origin) and human fibroblasts. The use of collagen as a matrix for migration in this report could contribute to the difference in phenotype. On collagen, cells treated with FAK and paxillin siRNAs exhibit smaller focal adhesions and a protrusive morphology (Yano et al., 2004), phenotypes that are distinct from those described for the *fak*$^{-/-}$ and *paxillin*$^{-/-}$ fibroblasts (Ilic et al., 1995; Hagel et al., 2002), albeit many analyses of these fibroblasts use fibronectin for adhesion. Notably, despite the enhanced extension of protrusions, HeLa cells with reduced FAK and paxillin are not highly polarized, and therefore might exhibit defects in directional migration. Thus, measuring random migration, as was done by Yano et al. (2004), could contribute to the unanticipated outcome of these new experiments because most reports implicating FAK as a positive regulator of migration have examined directional motility in response to chemotactic or haptotactic gradients (Ilic et al., 1995; Sieg et al., 2000).

Although several reports implicate paxillin as a positive regulator of motility (Hagel et al., 2002), overexpression of paxillin in several cell types is reported to impair haptotaxis (Yano et al., 2000). Interestingly, collagen was used as the haptotactic stimulus in this paper. In contrast to the results seen with inhibition of FAK and paxillin expression by siRNA, inhibition of p130cas, another focal adhesion–associated FAK-binding partner linked to promotion of migration (O'Neill et al., 2000), had no effect on migration, morphology, or formation of N-cadherin–containing adhesions (Yano et al., 2004). Hence, as described in this paper, a subset of focal adhesion–associated proteins are involved in the generation of this phenotype, possibly reflecting different roles for different FAK-containing complexes in the regulation of cell migration.

What is the mechanism through which FAK and paxillin inhibit motility and protrusion, and promote the formation of N-cadherin–containing adhesions? The phenotypes produced by inhibition of FAK and paxillin are reversed by coexpression of a dominant-negative Rac1 mutant and mimicked by expression of constitutively active Rac1, suggesting that FAK and paxillin may normally function in these scenarios to attenuate Rac1 signaling (Yano et al., 2004). Although there is no global change in the level of Rac1 activity in cells with reduced FAK or paxillin expression, experiments using a FRET-based Rac biosensor suggest local increases in activity in these cells, particularly at the periphery and at areas of cell–cell contact (Yano et al., 2004). How FAK might inhibit Rac1 activity is not clear, as previous papers suggest that FAK promotes Rac1 activation through a p130cas–Crk–Dock180 complex or potentially a paxillin–

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Abbreviation used in this paper: siRNA, small interfering RNA.

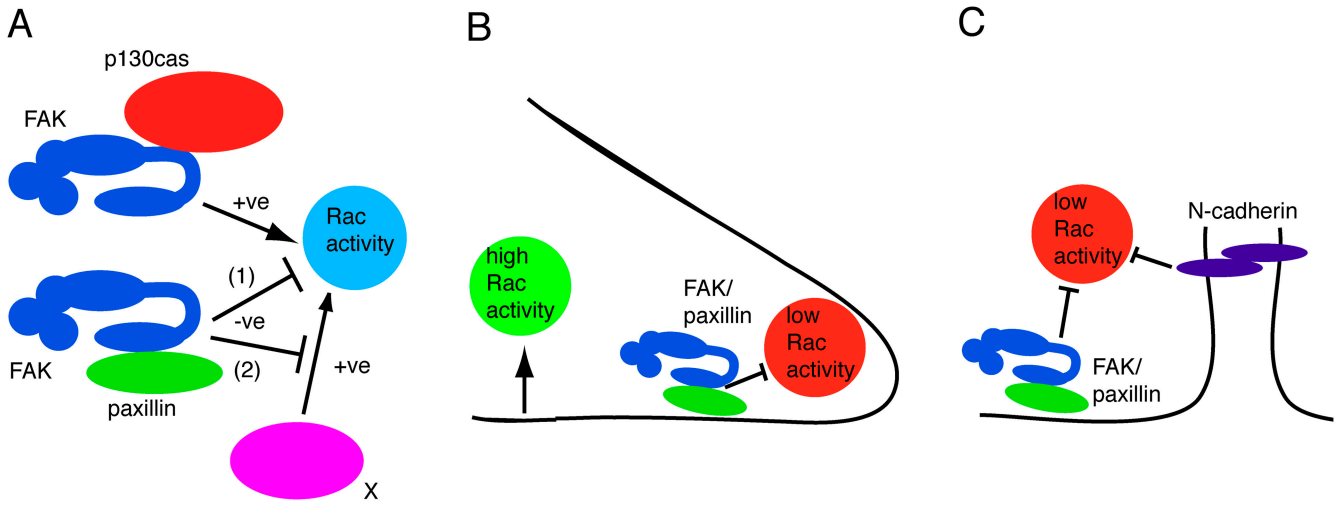

Figure 1. **Regulation of Rac activity by FAK paxillin.** (A) FAK/p130cas stimulates Rac activation, whereas FAK/paxillin may inhibit Rac activity. This may occur via direct targeting of Rac by FAK/paxillin-associated regulators of Rac (1). Alternatively, FAK/paxillin signaling may interfere via crosstalk with a distinct signaling pathway (X) that promotes Rac activation (2). (B) In this schematic of the edge of a cell, peripheral Rac activity is low (red), whereas nonperipheral Rac activity is high (green). At the periphery, FAK/paxillin may function to reduce Rac activity. This could be achieved by discrete relocalization of FAK/paxillin or spatially dependent modulation of the Rac-inhibiting activity of FAK/paxillin. (C) In this schematic of two cells in contact, FAK/paxillin signaling and N-cadherin signaling may both contribute to the reduced levels of active Rac near cell–cell junctions.

Crk–Dock180 complex (Hsia et al., 2003). Although FAK might recruit other proteins to directly target inactivation of Rac1 (e.g., a RacGAP), FAK might also indirectly impair activation of Rac1 by inhibiting a distinct signaling pathway that stimulates Rac1 activity (Fig. 1 A). Paxillin could simply function in regulating FAK localization as proposed (Yano et al., 2004), or paxillin could play a more direct role in regulation of Rac1 by recruiting a regulatory complex comprised of PKL, PIX, and PAK. Support for this latter mechanism comes from reports expressing paxillin mutants in CHO cells. Expression of a paxillin mutant that fails to associate with PKL produced a protrusive cellular morphology and elevated levels of active Rac1 after cell adhesion (West et al., 2001). In addition, this mutant caused increased random motility on fibronectin, but reduced rates of directional motility in a wound-healing assay. The similarity in phenotype induced by expression of this mutant and inhibition of paxillin by siRNA validates PKL and associates as candidates for negative regulation of motility by paxillin.

Tyrosine 861, a site of Src-dependent phosphorylation on FAK, was implicated in retarding motility (Yano et al., 2004). Phosphorylation of this site could regulate interactions with binding partners that function to inactivate Rac1 signaling, or alternatively, might contribute to localization or spatially regulated activation of FAK signaling (e.g., at the cell periphery), providing a mechanism for regulating biochemical events in a spatial manner (Fig. 1 B). VEGF-stimulated phosphorylation of tyrosine 861 promotes association of FAK with αvβ5 integrins in endothelial cells (Eliceiri et al., 2002). Although αvβ5 may not be relevant to the current paper, this finding presents an interesting paradigm for a mechanism regulating FAK function in a spatial fashion.

Another intriguing observation was the impaired recruitment of N-cadherin to sites of cell–cell contact when cells with reduced FAK/paxillin expression were plated at low density, although these cells could form N-cadherin–containing contacts when plated at high density. Further, FAK/paxillin was required for the maintenance of N-cadherin–containing cell–cell contacts in cells at the edge of wounds introduced into confluent monolayers. A local reduction in Rac1 activity at the region of cell–cell contact, which requires FAK and paxillin, may control the recruitment/maintenance of N-cadherin at these sites. The function of Rac1 at sites of cell–cell adhesion is complex. Although engagement of cell surface E-cadherin can stimulate activation of Rac1 (for review see Yap and Kovacs, 2003), engagement of N-cadherin is reported to reduce the activity of Rac1 (Charrasse et al., 2002). In a number of cases, Rac1 signaling is required for the assembly of cadherin into cell–cell adhesions. In keratinocytes, constitutively active Rac1 and dominant-negative Rac1, which functions to block endogenous Rac1, can produce the same phenotype, loss of E-cadherin from sites of cell–cell contact (Braga et al., 1999, 2000). In this scenario, the appropriate level of Rac1 activity is required for assembly and maintenance of E-cadherin–containing junctions. Intriguingly, different extracellular matrices can modulate the biological consequences of Rac activation by Tiam1, a guanine nucleotide exchange factor for Rac. On fibronectin and laminin Tiam1 promoted cell–cell adhesions in MDCK cells, whereas the same cells exhibited a motile phenotype when plated on collagen (Sander et al., 1998). As the N-cadherin analyses reported by Yano et al. (2004) were performed on collagen, the latter paradigm may be relevant given the correlation of elevated Rac1 activity at sites of cell–cell contact and the failure to assemble N-cadherin–containing junctions. At these sites, FAK and paxillin may function to initially reduce the level of Rac1 activity, but upon establishing homophilic interactions between N-cadherins on adjacent cells, it is likely that N-cadherin–mediated signaling also functions in dampening Rac1 activity in these regions of the cell (Fig. 1 C).

The Yano et al. (2004) paper is provocative for a number of reasons. It defines a novel role for FAK and paxillin in controlling the assembly of N-cadherin contacts. It also suggests FAK and paxillin may function in inhibiting cell motility in addition to their established functions in promoting migration. It is quite interesting that the same regulatory molecules could positively and negatively control migration in different contexts. Clearly, elucidation of the conditions dictating positive or negative regulation and the mechanism of action in these two scenarios will be high priorities in the near future.

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