Moreover Kirby *et al.*[6] have recently observed an EM for an unusually favourable intramolecular proton transfer to carbon in excess of 6×10^4 M. Detection of large EM values such as these improve the prospects for the rational design of efficient proton transfer catalysts, but also reveal deficiencies in the predictive understanding of this most fundamental and thoroughly studied of chemical processes.

Despite their simplicity and the wealth of data available for them, proton transfers remain a live issue for bio-organic chemistry and enzymology. In the past three years, a proposal of a proton transfer crane[7], a detailed analysis of the catalysis of malic acid formation by the enzyme fumarate[8], strong evidence for the intervention of quantum-mechanical tunnelling effects for a variety of enzyme-catalysed proton transfers[9], and conferences and reviews devoted to the structural enzymology[10] of proton transfer reactions all show the degree of current interest.

At least in principle, studies of catalytic antibodies should hold a mirror up to nature. For the important class of proton transfer reactions, Thorn *et al.* have shown us a first image. □

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CELL ADHESION

Cryptic sites in vinculin

Andrew P. Gilmore and Keith Burridge

FOR more than a decade there has been controversy over whether vinculin, an abundant cytoskeletal protein concentrated at sites of cell adhesion, interacts directly with actin. Vinculin is found in cell–extracellular matrix adhesions involving integrins (focal adhesions) and in cell–cell adhesions mediated by cadherins (adherens junctions). Both of these regions are major sites for the attachment of actin filaments to the plasma membrane, and current evidence suggests that vinculin is a big player in this attachment. The controversy is now resolved, with an interesting twist, by Johnson and Craig on page 261 of this issue[1]. These authors confirm that vinculin does contain an actin-binding domain, but show that this site is normally masked in the purified protein.

Given vinculin's distribution in cell adhesions, it was assumed from the time of its discovery that its job is to link actin to the membrane. This assumption seemed to be confirmed when several labs demonstrated an interaction between vinculin and actin. Not long after, however, it was shown that most of the actin-binding activity in vinculin preparations was due to contaminants, which could be removed by further purification. One of these contaminants was identified as a fragment of tensin, another focal adhesion protein that binds to vinculin and caps actin filaments. In addition vinculin was found to bind two other prominent focal adhesion proteins, α-actinin and talin, both of which interact with integrins and actin *in vitro*. In many models of focal adhesions, vinculin's function was relegated to that of linker between talin and α-actinin. Nevertheless, Jockusch's group, which first identified the interaction with actin, has continued both to maintain that this interaction occurs and to provide evidence for it. Most recently, getting around the criticism of contaminants, they have used bacterially expressed fragments to show that the carboxy-terminal tail of vinculin binds actin[2]. This finding was convincing, but difficult to reconcile with the absence of binding found by others with intact vinculin.

Vinculin has two principal domains, an amino-terminal globular head and an elongated carboxy-terminal tail (in electron microscopic images, it looks like a balloon on a string). Johnson and Craig previously identified an intramolecular interaction between the head and tail domains of vinculin, and showed that it decreases the affinity of vinculin for talin[3]. This study laid the groundwork for their reinvestigation of vinculin's interaction with actin. In agreement with the work of Jockusch's group, they have identified an actin-binding site in the tail of vinculin, but show that the head–tail interaction normally masks the site in the purified protein. More than simply resolving a controversy, this work raises the question of whether the site is unmasked in focal adhesions and, if so, how the process might be regulated. It is easy to envisage how a conformational change that promotes interaction of focal adhesion proteins could be critical to focal adhesion assembly. An appealing model is presented in Johnson and Craig's Fig. 3 on page 264.

There is considerable interest in signalling pathways initiated in response to cell adhesion that, among other consequences, may contribute to the development of focal adhesions. An early event, following adhesion to extracellular matrix proteins, is activation of the focal adhesion kinase[4] and the tyrosine phosphorylation of a number of focal adhesion components. Treating cells with tyrosine kinase inhibitors blocks focal adhesion assembly[5], but the link between tyrosine phosphorylation and assembly is yet to be resolved. Could tyrosine phosphorylation somehow affect the head–tail interaction in vinculin? In normal cells the level of phosphotyrosine in vinculin is minimal and so it is unlikely that the actin-binding site in vinculin is unmasked by direct tyrosine phosphorylation of vinculin itself. However, two of the proteins that become tyrosine phosphorylated in response to adhesion, paxillin and tensin, bind vinculin. Significantly, paxillin's binding site on vinculin has been mapped to the tail domain[6], raising the interesting and testable idea that tyrosine phosphorylated paxillin (or some other protein) might bind to vinculin and thereby expose its actin-binding site.

Another signalling molecule implicated in focal adhesion assembly is the small GTP-binding protein, rho. Microinjection of activated rho into quiescent cells with diminished focal adhesions and stress fibres stimulates the reformation of these structures[7]. Most recently, rho has been shown to regulate phosphatidylinositol-4-phosphate 5-kinase, leading to the synthesis of phosphatidylinositol-4,5-bisphosphate ($PtdInsP_2$)[8]. Several actin-binding proteins are regulated by $PtdInsP_2$ and an interaction of vinculin with $PtdInsP_2$ has been reported[9], again raising the possibility that $PtdInsP_2$ or related phospholipids may regulate the accessibility of the cryptic site in vinculin.

Progress in understanding the organization and assembly of focal adhesions has been slow. Johnson and Craig's identification of a cryptic actin-binding site in vinculin may be a missing piece of the puzzle. If this discovery leads to experiments that connect adhesion-mediated signalling pathways with the assembly of focal adhesions, then the significance of this work will go far beyond the resolution of the long-standing controversy of whether vinculin does or does not bind actin. □

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