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To B or Not to B: PIP2 Answers the Question

In a recent issue of *Molecular Cell*, Papayannopoulos et al. (2005) show that N-WASP activation of actin-related protein2/3 (Arp2/3) is ultrasensitive to PI(4,5)P_2 concentration. We discuss how interactions between basic regions in proteins and negatively charged membranes can produce sharp on-off switches that may regulate biological activity.

N-WASP is a key regulator of actin polymerization, which is controlled by N-WASP-mediated inhibition of Arp2/3 (see Figure 1A). N-WASP contains an N-terminal basic (B) motif, a G protein (GTP-Cdc42) binding domain (GBD), and a VCA domain that binds to and activates Arp2/3 (Higgs and Pollard, 2000; Prehoda et al., 2000; Rohatgi et al., 2000). Inactivation involves autoinhibitory interactions between GBD and the VCA domain and between the B motif and Arp2/3. The constitutively active VCA domain is released when GTP-Cdc42 binds to GBD and when phosphatidylinositol 4,5-bisphosphate (PIP_2) interacts with the B motif. The major new finding by Papayannopoulos et al. (2005) is that, in the absence of Cdc42, N-WASP activation of Arp2/3 is highly sensitive to the PIP_2 composition of the membrane. The activation threshold corresponds to a small fold change in PIP_2 surface density (0.5–1.5 fold), suggesting that subtle changes in PIP_2 concentration in the plasma membrane may induce a "switch" for N-WASP-mediated Arp2/3 actin polymerization. Extrapolation of the in vitro results should be made with caution. Nevertheless, it is intriguing that a lipid present at relatively low levels (< 10%) may provide such a sharp activation switch.

Both the binding of N-WASP to phospholipid vesicles and the N-WASP-mediated activation of actin polymerization were measured as a function of mole percent PIP_2 in membranes. Membrane binding and actin polymerization are represented by sigmoidal curves, reminiscent of the well-known example of cooperativity observed in the binding of oxygen to hemoglobin. In the current study, the authors calculate an apparent Hill coefficient for membrane binding of the B motif of $\sim$3 and one for activation of Arp2/3 of $\sim$20. In both cases, the interaction of the B motif with PIP_2 plays a major role in the observed cooperativity.

There have been extensive biophysical and computational studies of the adsorption of basic peptides and proteins to membranes containing acidic phospholipids (see, for example, Ben-Tal et al., 1996; Murray and Honig, 2002). Binding is driven by electrostatic attraction between a positively charged peptide and a negatively charged membrane (see Figure 1B). However, basic peptides do not "bind" to phospholipids in the usual sense: the interaction is nonspecific and the peptides diffuse in a two-dimensional plane parallel to the membrane surface. In the case of hemoglobin, the cooperativity due to the binding of oxygen results from allosteric conformational changes in hemoglobin. There is no obvious basis for this type of effect in the case of the adsorption of basic peptides to membrane surfaces.

Mosior and McLaughlin (1992) have provided a model that accounts for the first type of cooperativity observed by Papayannopoulos et al. (2005), i.e., the sigmoidal dependence of the "binding" of basic peptides to membranes as a function of acidic phospholipid. Two distinct mechanisms come into play. The first involves a reduction of dimensionality effect in which the attraction of the peptide to the "first" acidic lipid increases the proximity of the peptide (or B motif) to the membrane so that the apparent association constants of the remaining basic residues are now stronger. The second mechanism results from the fact that the concentration of bound peptide of charge q is related to the electrostatic potential of the membrane, ϕ, through the Boltzmann factor, $\exp(-qF\phi/RT)$, where F is the Faraday constant. Note that as the magnitude of ϕ increases, the concentration of bound peptide will increase exponentially. Since ϕ is in turn related to the amount of acidic lipid, i.e., the higher the PIP_2 concentration the more negative the membrane potential, one expects the apparent cooperativity to increase, as observed. Following Mosior and McLaughlin (1992), we calculated the membrane association of peptides based on the B motif constructs examined by Papayannopoulos et al. (2005) as a function of PIP_2 concentration. As evident from Figure 1C, apparent Hill coefficients of about 3-6 are consistent with a model based solely on reduction of dimensionality and nonspecific electrostatics. Moreover, the curves become steeper with increasing peptide charge, consistent with the experimental observations.

Rather more surprising is the second type of cooperativity observed, i.e., the much sharper transition for N-WASP-activated actin polymerization (apparent Hill coefficient of $\sim$20). However, greater cooperativity is, in fact, expected if the off/on activation switch occurs at more negative values of the membrane potential than does binding alone and, therefore, at larger PIP_2 concentrations. Why should a much larger membrane potential (and consequently more PIP_2) be required to disrupt the B motif-Arp2/3 inhibitory interaction than is required to localize the isolated B motif to the membrane surface? It is interesting in this regard that Arp2/3 has large patches of negative potential on its surface, one of which may compete with the negatively charged membrane for the B motif. Thus, the switch-like behavior of Arp2/3 regulation may be due to an electrostatic competition of two negatively charged surfaces (Arp2/3 versus membrane) for a positively charged sequence (the B motif). The inhibition cannot be relieved until there is enough PIP_2 to produce a local membrane potential (Figure 1B) strong enough to outcompete the inhibitory protein/protein interaction (Figure 1A). The stronger potential required for activation (versus binding) results in greater nonlinearities through the Boltzmann factor, described above, and conse-

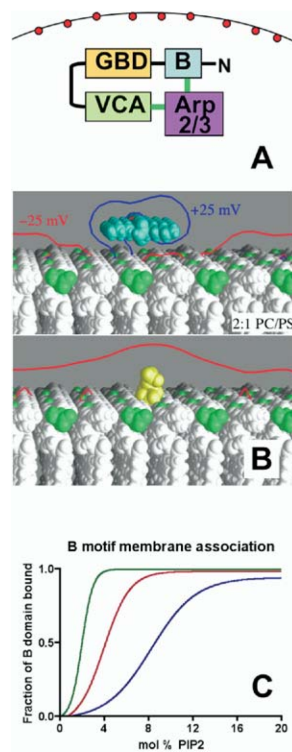

Figure 1. Models of the N-WASP, B Domain, and Calculated Binding Curves

(A) Schematic diagram of the components of N-WASP activation. Black lines connect N-WASP domains while green lines denote inhibitory interactions that are broken upon activation. PIP_2 molecules in the plasma membrane are shown as red circles.

(B) Calculated electrostatic potentials generated by acidic phospholipids and basic peptides (10). Upper panel: Heptalysine adsorbed to a PC/PS membrane in 0.1 M KCl. Lower panel: PIP_2 enhances the negative potential of the membrane in its vicinity.

(C) Fraction peptide bound to a membrane, described as a plane of smeared negative charge (8), as a function of PIP_2 concentration. Blue, red, and green curves denote calculations for peptides with net charges of +7, +10, and +14, as considered by Papayannopoulos et al. (2005); the calculations yield apparent Hill coefficients of 3.6, 3.7, and 5.6, respectively.

quently both a sharper transition and the dramatically higher apparent Hill coefficient (∼20 for activation versus ∼3 for membrane binding only).

A similar mechanism may be operative in the activation of conventional protein kinase C (cPKC) isoforms where binding of the N-terminal basic pseudo-substrate in the kinase catalytic cleft is associated with its inactive form (Newton, 1993). In response to an increase in intracellular calcium (which localizes the protein near the membrane surface through its C2 domain), the membrane can then compete with the kinase domain for the pseudo-substrate, leading to the interfacially active form of the enzyme. Thus, in both N-WASP and PKC, a competition is set up between the binding of a basic motif to its parent protein and the binding of the motif to acidic phospholipids. Moreover, the PIP_2-mediated activation of N-WASP is likely coupled to the activation of cPKC: cPKC phosphorylation of its major substrate, MARCKS, is postulated to release laterally sequestered pools of PIP_2 in the plasma membrane (Gambhir et al., 2004), giving rise to local bursts of PIP_2, which may produce a strong enough potential to mediate the switch-like activation of N-WASP.

The sharp activation transitions observed by Papayannopoulos et al. (2005) point to a new kind of "electrostatic switch" that can function in signaling pathways. We have seen here that such a switch is consistent with nonspecific electrostatic interactions that have been shown to play a crucial role in many signaling events (see, for example, Johnson and Cornell, 1999). Other kinds of electrostatic switches, involving the interaction of proteins with acidic phospholipids, have been discussed (see, for example, Murray and Honig, 2002). It is remarkable how biological macromolecules have learned to exploit "simple" electrostatic interactions in such novel ways in the regulation of highly complex physiological processes.

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