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Opening the GAP

Diacylglycerol is a lipid second messenger involved in cell signaling and phorbol esters are natural products that mimic that action. Diacylglycerol mediates signaling from a variety of hormones through G protein-coupled receptors, from growth factors via receptor tyrosine kinases, and many other intra- and extracellular agents. The binding of diacylglycerol or phorbol esters to the protein kinase C homology 1 (C1) domain of a target protein results in its translocation to the membrane and subsequent allosteric activation. The mechanism for this lipid-mediated membrane association has been examined by studies of C1 domains from the protein kinase C (PKC) family. Several other C1-containing proteins including the α - and β -chimerins have recently been identified.

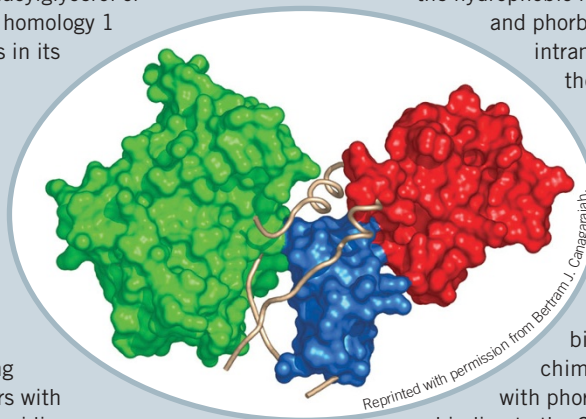
The chimerins are GTPase-activating proteins (GAP) that bind phorbol esters with nanomolar affinity in the presence of acidic phospholipids. β 2-chimerin is composed of three conserved domains: an N-terminal SH2 domain for phosphotyrosine binding to an unknown partner, a C1 domain, and a C-terminal Rac-specific GAP domain. Rac is a small G protein that regulates an array of cellular activities, including the cell cycle, actin dynamics and transcription. Rac binding to RacGAP promotes its GTPase activity. To understand what conformational changes occur upon lipid binding and how this leads to Rac activation, Hurley and colleagues have solved the structure of β 2-chimerin (*Cell*, in the press, 2004).

The three domains of β 2-chimerin are arranged with the C1 (blue) sandwiched between RacGAP (green) and SH2 (red). The C1 and RacGAP domains have the canonical fold of their representative family members, while the C-terminal part of the SH2 domain resembles that of the adaptor protein APS.

The N-terminus (beige wire) runs the length of the protein, covering the RacGAP active site and the lipid-binding pocket of C1. The C1 domain makes extensive contacts with the SH2 and RacGAP domains, and the residues in C1 that form the basic face for putative membrane association are solvent-exposed. However, the hydrophobic residues implicated in diacylglycerol and phorbol ester binding are buried and form intramolecular interactions with the rest of the protein. This implies that a dramatic conformational change is required prior to the binding of lipid second messengers to the C1 domain. Mutational analysis indicates that the destabilization of C1 interactions with the remainder of the protein favors the conformational changes that occur upon membrane binding. This suggests that the β 2-chimerin intramolecular contacts compete with phorbol ester and phospholipids for binding to the C1 domain.

Modeling of the β 2-chimerin structure with that of Rac-GDP, combined with comparison to the liganded p50RhoGAP domain structure, shows how β 2-chimerin may activate Rac. When lipid binding occurs, a large conformational change causes the N-terminus of β 2-chimerin to move away from the RacGAP domain, allowing Rac to bind. The RacGAP domain interacts directly with the Rac active site, stabilizing its transition state for GTP hydrolysis. In essence, the N terminus of β 2-chimerin serves as an inhibitory domain similar to the N-terminal pseudosubstrate sequences used by the PKCs. However, whereas the PKCs have two C1 domains—one specialized for translocation and the other for activation— β 2-chimerin has a more rudimentary system whereby both functions are accomplished by a single C1 domain. It is remarkable that proteins with such differing targets would have similar modes of activation.

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