PROTEIN BIOCHEMISTRY

Protein Prenylation, et cetera: Signal Transduction in Two Dimensions

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Most eukaryotic proteins are decorated with chemical groups—phosphates, methyl groups, sugars, or lipids—during or after their translation from mRNA. These extra functional groups have various purposes, often serving as switches or localization signals. One kind of lipid modification is protein prenylation, in which 15-carbon farnesyl or 20-carbon geranylgeranyl groups are attached to the COOH-terminus of a protein, followed by other modifications (proteolysis, methylation, and palmitoylation) (*1*, *2*). Two reports on pages 1796 and 1800 of this issue illuminate this process. Park *et al.* (*3*) report the x-ray structure of one enzyme, mammalian protein farnesyltransferase (FTase), that links the farnesyl group to proteins, and Boyartchuk *et al.* (*4*) identify two genes in yeast that are responsible for a later modification step, proteolytic removal of the last three amino acids of the prenylated protein.

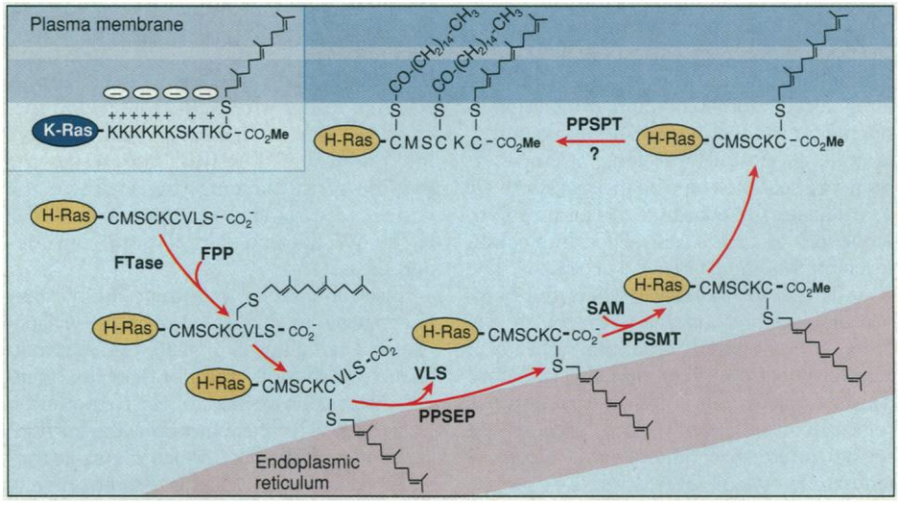
Most prenylated proteins are members of signal transduction cascades—for example, the γ-subunits of heterotrimeric guanosine triphosphate (GTP)–binding proteins (G proteins) (*5*) and virtually all members of the Ras superfamily of proteins (*2*, *6*, *7*). Interest in protein prenylation has escalated in recent years because of the importance of this modification for the function of Ras proteins, GTP–binding proteins that when mutated cause some cancers. Indeed, farnesylation of H-, K- and N-Ras is essential for the ability of oncogenic mutants of these proteins to transform cells (*8*). This is quite an important result, when one considers that about 30% of established tumor cell lines contain mutationally activated Ras proteins. Even though important proteins other than Ras are farnesylated in cells, some protein FTase inhibitors remarkably shrink tumors in animals to an undetectable size, and yet the animals show no significant toxicity after weeks or months of exposure (*8*).

Farnesylation is catalyzed by FTase (*9*), the structure of which is now revealed (*3*). This enzyme uses farnesyl diphosphate (FPP) as the prenyl donor. Two other enzymes, protein geranylgeranyltransferase type I (GGTase-I) and GGTase-II, attach one or two 20-carbon prenyl groups, respectively, to specific proteins (*10*). The structure of FTase provides a clear molecular picture of why this enzyme transfers farnesyl groups much better than geranylgeranyl groups. The binding pocket for FPP has a well-defined length, and binding of geranylgeranyl diphosphate (GGPP) at this site would leave the electrophilic carbon too far from the attacking sulfhydryl group of the prenyl acceptor. Consistent with this idea is the fact that FTase binds FPP only 30 times as tight as it does GGPP (*11*). In contrast, GGTase-I binds GGPP 300 times as tight as it does FPP (*11*), likely because GGTase-I has a longer binding site that allows additional favorable interactions with the extra isoprene unit of GGPP. The FTase crystal structure will help guide medicinal chemists as they develop more potent and selective FTase inhibitors.

Because prenylated signal transduction proteins function on cell membranes, they have been thought to be anchored to the membranes by hydrophobic prenyl groups. Evidence for such tethering exists in some cases (*12*). Nevertheless, there may be more to prenylation than membrane anchoring (and more to membrane anchoring than prenylation). Some prenylated proteins are in the plasma membrane and others are bound to internal cell membranes. How is this accomplished? What are the functions of the additional modifications of prenylated proteins? It has yet to be determined why some proteins are modified by farnesylation, others by geranylgeranylation, and still others by double geranylgeranylation (*13*).

Why bring signal transduction proteins to membranes? After all, intracellular regions of transmembrane receptors could interact with cytosolic components. One idea is that colocalization of components onto a two-dimensional surface may enhance their interactions with each other by at least a factor of 10^6, mainly as a result of the high local concentrations of proteins tethered to membranes (*14*). Colocalization may thus dictate specificity among signal transduction pathways.

How does prenylation promote membrane binding of proteins? H-Ras that has undergone COOH-terminal farnesylation, proteolysis, and methylation (see figure) is mainly cytosolic, and a final modification step of palmitoylation is required for Ras to bind to the plasma membrane (*15*, *16*). The same is true for N-Ras, which also is palmitoylated, but not for K-Ras. K-Ras does not contain a palmitoylation site but rather a cluster of eight basic residues that may electrostatically interact with acidic phospholipids on the in-



The making of modified Ras. Protein FTase transfers the farnesyl group from farnesyl diphosphate (FPP) to the SH group of a cysteine near the COOH-terminus of the protein. The COOH-terminal tripeptide Val-Leu-Ser is removed by a prenyl protein–specific endoprotease (PPSEP) in the endoplasmic reticulum, and then a prenyl protein–specific methyltransferase (PPSMT) donates the methyl (Me) group from *S*-adenosylmethionine (SAM) to the COOH-terminal *S*-farnesylated cysteine. The final step is the attachment of palmitoyl groups to the cysteines near the farnesylated COOH-terminus. A prenyl protein–specific palmitoyltransferase (PPSPT) is shown in the plasma membrane, although the existence and location of such an enzyme is not yet established. K-Ras is farnesylated, proteolyzed, and methylated, as is H-Ras, but may interact with the plasma membrane electrostatically and by insertion of the farnesyl group into the membrane.

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ner leaflet of the plasma membrane (see figure) (*15*, *17*). Palmitoylation of yeast Ras2p is required for membrane binding and glucose signaling (*18*). So why farnesylate H-Ras if palmitoylation is required for membrane binding? Perhaps palmitoylation alone is not sufficient to anchor Ras to membranes. This notion has not been tested directly because in cells farnesylation is a prerequisite for palmitoylation (*6*, *7*). Palmitoylation of H-Ras may occur only in plasma membranes by a putative palmitoyltransferase that is bound to the plasma membrane. Farnesylation may bring a finite amount of H-Ras to all cellular membranes, and palmitoylation may then be required to trap it in the plasma membrane. Our understanding of the enzymology of protein palmitoylation has only just begun (*19*); however, we do know that H-Ras palmitoylation, like G protein α-subunit palmitoylation, is a reversible event and so may regulate signal transduction (*20*). Some transfection studies have led to the suggestion that H-Ras palmitoylation is not required for cellular transformation (*6*), but studies in *Xenopus* oocytes with more physiological amounts of H-Ras indicate that Ras activates oocytes very poorly, if at all, unless it is palmitoylated (*16*).

In addition to palmitoylation, prenylated proteins are subject to COOH-terminal proteolysis and methylation. Are these modifications necessary for function of the protein? In this context, the new studies in yeast by Boyartchuk (*4*) are providing some insights. Two genes, *RCE1* and *AFC1*, are responsible for COOH-terminal proteolysis of prenylated proteins in yeast. In yeast that lack these functional proteases, Ras2p, which normally localizes to the plasma membrane, mislocalizes to the interior of the cell, at least when overexpressed. Loss of proteolysis reduces but does not eliminate Ras2p function in yeast expressing either high or endogenous levels of the protein. In *Xenopus*, proteolysis and methylation are required for palmitoylation, membrane binding, and the function of Ras (*21*). These studies suggest that the prenyl protein–specific protease and methyltransferase, like FTase, may be good targets for antioncogenic therapeutics, especially because yeast lacking prenyl protein–specific protease activity are viable.

References

1. J. A. Glomset, M. H. Gelb, C. C. Farnsworth, *Trends Biochem. Sci.* **15**, 139 (1990).
2. F. L. Zhang and P. J. Casey, *Annu. Rev. Biochem.* **65**, 241 (1996).
3. H.-W. Park, S. R. Boduluri, J. F. Moomaw, P. J. Casey, L. S. Beese, *Science* **275**, 1800 (1997).
4. V. L. Boyartchuk, M. N. Ashby, J. Rine, *ibid.*, p. 1796.
5. H. K. Yamane *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5868 (1990); S. M. Mumby, P. J. Casey, A. G. Gilman, S. Gutowski, P. C. Sternweis, *ibid.*, p. 5873; R. K. Lai, D. Perez-Sala, F. J. Canada, R. R. Rando, *ibid.*, p. 7673.
6. J. F. Hancock, A. I. Magee, J. E. Childs, C. J. Marshall, *Cell* **57**, 1167 (1989).
7. P. J. Casey, P. A. Solski, C. J. Der, J. E. Buss, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8323 (1989); W. E. Schafer *et al.*, *Science* **245**, 379 (1989).
8. K. S. Koblan *et al.*, *Biochem. Soc. Trans.* **24**, 688 (1996).
9. Y. Reiss, J. L. Goldstein, M. C. Seabra, P. J. Casey, M. S. Brown, *Cell* **62**, 81 (1990).
10. K. Yokoyama and M. H. Gelb, *J. Biol. Chem.* **268**, 4055 (1993); J. F. Moomaw and P. J. Casey, *ibid.* **267**, 17438 (1992); M. C. Seabra, J. L. Goldstein, T. C. Sudhof, M. S. Brown, *ibid.*, p. 14497.
11. K. Yokoyama, K. Zimmerman, J. Scholten, M. H. Gelb, *ibid.* **272**, 3944 (1997).
12. J. A. Glomset and C. C. Farnsworth, *Annu. Rev. Cell Biol.* **10**, 181 (1994).
13. C. C. Farnsworth *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6196 (1991).
14. M. H. Gelb and O. G. Berg, unpublished data.
15. J. F. Hancock, K. Cadwallader, C. J. Marshall, *EMBO J.* **10**, 641 (1991).
16. T. Dudler and M. H. Gelb, *J. Biol. Chem.* **271**, 11541 (1996).
17. F. Ghomashchi, X. Zhang, M. H. Gelb, *Biochemistry* **34**, 11910 (1995).
18. S. Bhattacharya, L. Chen, J. R. Broach, S. Powers, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2984 (1995).
19. L. Liu, T. Dudler, M. H. Gelb, *J. Biol. Chem.*, in press.
20. P. T. Wilson and H. R. Bourne, *ibid.* **270**, 9667 (1995).
21. T. Dudler and M. H. Gelb, unpublished data.

LIQUID CRYSTALS

Nematic Emulsions

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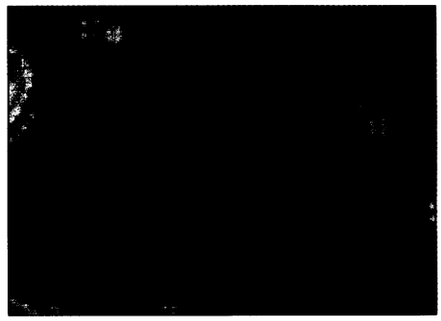
Emulsions are dispersions of liquid droplets, hundreds of nanometers to a micrometer in size, in a continuous liquid solvent. A typical example would be a dispersion of oil droplets in water (oil-in-water emulsion) or a dispersion of water droplets in an oil (water-in-oil emulsion). Many kinds of emulsions are encountered in the food or cosmetics industries, so understanding their behavior is of both scientific and technological importance. In general, emulsions are only metastable, and the droplets tend to coalesce. A large part of the research on emulsions (*1*) is thus devoted to the stability of the dispersion, to the monitoring of the interactions between droplets, and to the tailoring of surface active agents that prevent the rupture of a solvent film between two droplets. Emulsions stable over days or more are quite common. More recent work (*2*) deals with model emulsions with a monodisperse droplet size distribution, with the packing of droplets in a dense emulsion, or with emulsions having specific properties. An example would be magnetic emulsions, where the droplets are made of a magnetic fluid. Under the action of a magnetic field, the ferrofluid becomes polarized and each droplet acquires a dipole moment parallel to the field. The attractive interactions between the dipole moments induces the formation of chains of droplets.

On page 1770 of this issue, Poulin *et al.* (*3*) report a new type of emulsion in which the continuous solvent phase is not an isotropic liquid but a nematic liquid crystal, the dispersed phase being water droplets. The nematic liquid-crystal order parameter is its director field, which gives the local average orientation of the molecules. At the surface of each water droplet, the nematic director has a preferential orientation, and thus, the presence of the water droplets perturbs the nematic ordering. The distortion of the nematic field costs elastic energy and induces an interaction between the droplets. The nematic interaction between droplets depends not only on the bending constants of the nematic liquid crystal that measure the energy cost of the director distortion but also on the boundary conditions given by the orientation of the director at the surface of each droplet and at the external surface of the nematic liquid (which imposes the director field in the absence of the water droplets). A wide variety of behaviors can be expected when all of these parameters are varied.



Stalking the wild hedgehog. Viewed between crossed polarizers, the water droplets dispersed in liquid-crystal solvent exhibit unusual colloidal interactions. Black regions are water; colored regions are nematic liquid crystal. The orientation of the liquid crystal on the water droplet leads to the formation of topological defects called hedgehogs. A hedgehog (hyperbolic hedgehog) is seen in between two neighboring water droplets. [Reprinted from Poulin *et al.* (*3*)]

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