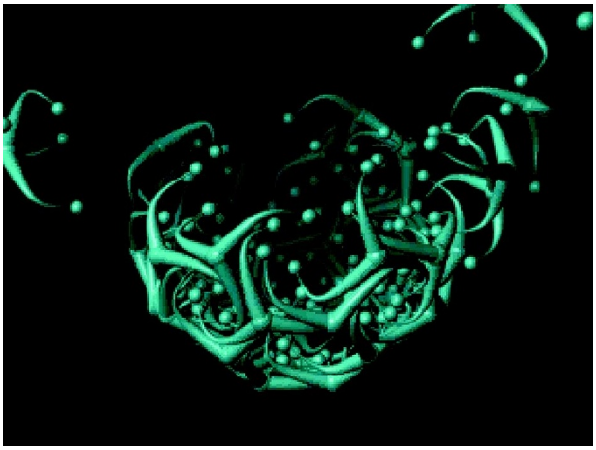
Clathrin-coat formation in time and space



Clathrin-coated vesicles mediate membrane-protein sorting during endocytosis and organelle biogenesis. Although it is known that clathrin triskelions self-assemble into many polyhedral lattice designs, including a soccer-ball-like shape, it's been difficult to imagine what this process looks like on a molecular level.

Tom Kirchhausen (Harvard Medical School) has added an extra dimension to our understanding of this process by modelling clathrin-coat formation in time and space. With the help of Allison Bruce (Genentech) he has produced a striking movie in which we, the viewers, observe the formation of a clathrin coat from the perspective of the plasma membrane. Taking advantage of recent X-ray crystallographic and electron cryomicroscopy data, he has modelled individual clathrin triskelions as rigid bodies, entering (or exiting) the coat by simple rotations and translations. The result is an elegant and artistic depiction of coated-vesicle formation. The image at the left represents a vesicle midway through the assembly process. There is free access to the movie at http://www.hms.harvard.edu/news/clathrin.

stress, and immunopurified Akt phosphorylates eNOS. They establish that Akt phosphorylates eNOS selectively at serine residue 1,177 (using the numbering of ref. 2).

Zeiher and colleagues[2] and Sessa and co-workers[1] also identify several remarkable properties of phosphorylated eNOS. First, eNOS derived from cells that are expressing a constitutively active Akt mutant exhibits greater catalytic activity than eNOS derived from cells with basal Akt levels[1]. Second, although unphosphorylated eNOS depends on calcium for activity, eNOS that has been phosphorylated by Akt[1], or an eNOS in which serine 1,177 is replaced with an aspartate to mimic phosphorylation[2], is less dependent on intracellular calcium.

Sessa and colleagues[1], who looked at the activation of eNOS by PI(3)K/Akt following cell treatment with vascular endothelial growth factor rather than shear stress, studied the calcium dependence of eNOS in cultured endothelial cells infected with adenoviral constructs expressing active and dominant-negative forms of Akt. In cells infected with constitutively active Akt, eNOS retains catalytic activity even at calcium concentrations equivalent to basal levels (100 nM). Although eNOS retains some calcium dependence, this may be due to the presence of residual unphosphorylated eNOS in the expression system used. Zeiher and colleagues[2] find that the serine 1,177→aspartate eNOS mutant exhibits near maximal levels of NO production even in the presence of just 10 nM cytosolic calcium. Phosphorylation-mediated formation of a calcium-independent enzyme would lead to constitutive NO production, accounting for basal vasomotor tone.

Vasodilation involves NO-induced relaxation of smooth-muscle cells, and so NO must diffuse out of the endothelial cell in which it is produced to the smooth-muscle cells. eNOS is dually acylated by palmitate and myristate, and these modifications are required for it to interact with caveolae — small invaginations — on the endothelial-cell plasma membrane[5,6]. The clustering of eNOS at caveolae, which are enriched in signalling molecules, may enable eNOS to be efficiently regulated by Akt. Indeed, Sessa and co-workers[1] show that Akt phosphorylates palmitoylated eNOS only, so the targeting of eNOS to caveolae may be a prerequisite for its phosphorylation and activation.

A second function of eNOS clustering at caveolae may be to enhance the effects of NO, which must travel from the endothelial plasma membrane through the smooth-muscle-cell membrane to the smooth-muscle-cell cytosol. Being highly reactive, NO is readily inactivated by superoxide or glutathione. Clustered eNOS might produce concentrated sources of NO that saturate these inactivation mechanisms, allowing for efficient relaxation of vascular smooth muscle. A drawback of this model is that the caveolar protein caveolin actually inhibits eNOS activity[7,8], probably by reducing its affinity for calmodulin. The new results[1,2] raise the possibility that eNOS phosphorylated on serine 1,177 might not be inhibited by its association with caveolin.

Does the PI(3)K/Akt pathway regulate NO production outside the endothelium? Sessa and colleagues[1] show that membrane targeting of the neuronal isoform of NOS (nNOS), achieved by attaching it to a myristoylation sequence, leads to its activation by Akt as well, suggesting a function for Akt in regulating NO synthesis in neurons. Indeed, the phosphorylation site in eNOS is conserved in the neuronal isoform, so a signalling pathway that leads to phosphorylation of nNOS may exist too, perhaps mediated by Akt or a kinase with similar specificity.

A large body of literature suggests that NO has a role in the long-term regulation of blood vessels. But many researchers have questioned whether NO can in fact be responsible for these effects, mainly because known mechanisms for activation of eNOS do not adequately explain them. The new findings provide a molecular explanation for these physiological observations, and indicate possible strategies for therapeutic intervention. Numerous groups have developed models in which transfer of the eNOS gene is used to improve vascular perfusion through coronary and femoral arteries[9]. It might be worthwhile to attempt such gene transfer with a form of eNOS in which serine 1,177 is mutated to aspartate, to provide constitutive NO synthesis. Another potential approach involves the development of drugs that activate Akt. Such drugs might form the basis for new antihypertensive agents with anti-atherogenic properties. □

Solomon H. Snyder and Samie R. Jaffrey are at The Johns Hopkins University School of Medicine, Departments of Neuroscience, Pharmacology and Molecular Sciences, and Psychiatry, 725 N. Wolfe Street, Baltimore, Maryland 21205, USA.
e-mail: ssnyder@bs.jhmi.edu

1. Fulton, D. *et al. Nature* **399,** 597–601 (1999).
2. Dimmeler, S. *et al. Nature* **399,** 601–605 (1999).
3. Busse, R. & Fleming, I. *J. Vasc. Res.* **35,** 73–84 (1998).
4. Dimmeler, S. *et al. Circ. Res.* **83,** 334–341 (1998).
5. Shaul, P. W. *et al. J. Biol. Chem.* **271,** 6518–6522 (1996).
6. Garcia-Cardena, G. *et al. Proc. Natl Acad. Sci. USA* **93,** 6448–6453 (1996).
7. Michel, J. B., Feron, O., Sacks, D. & Michel, T. *J. Biol. Chem.* **272,** 15583–15586 (1997).
8. Garcia-Cardena, G. *et al. J. Biol. Chem.* **272,** 25437–25440 (1997).
9. Von der Leyen, H. E., Mann, M. J. & Dzau, V. J. *Semin. Interv. Cardiol.* **1,** 209–214 (1996).