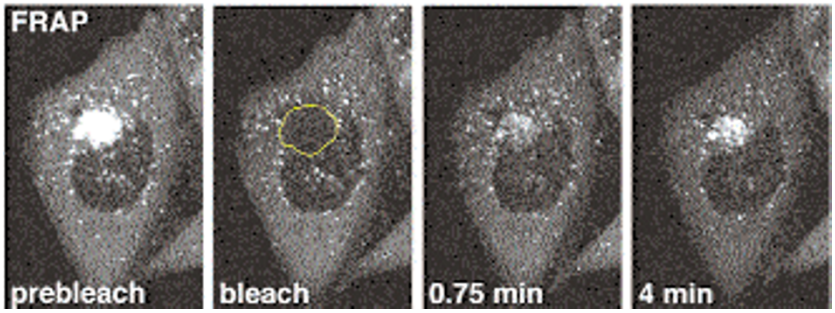
Shedding new light on coatomer dynamics

The use of fluorescent proteins is expanding to include increasingly sophisticated techniques. Fluorescent protein tags, in combination with live cell imaging and photobleaching, offer researchers the opportunity to study protein dynamics in living cells, and the observations are sometimes surprising. In a recent issue of *Nature*, Presley, *et al.* (*Nature* **417**, 187–193; 2002) used fluorescence loss in photobleaching (FLIP) and fluorescence recovery after photobleaching (FRAP) to investigate the *in vivo* dynamics of the cytosolic coat protein, coatomer or COPI.

Cytosolic coat proteins bind reversibly to organelle membranes and mediate vesicle formation that is necessary to transfer cargo proteins between organelles within the cell. Coatomer binding is mediated by the GTP-binding protein Arf1, and budding vesicles are transported between the Golgi apparatus and the endoplasmic reticulum (ER). In the generally accepted model, this process is thought to occur in a stepwise manner. Cytoplasmic Arf1–GTP binds to the Golgi membrane and recruits cytoplasmic coatomer. Coatomer polymerizes into an electron-dense coat, shaping the membrane into a coated bud and ultimately giving rise to the coated vesicle. The coated vesicle then 'uncoats' or detaches from the membrane in a process that is thought to be directly coupled with Arf1–GTP hydrolysis to Arf1–GDP.

In separate experiments using coatomer or Arf1 labeled with fluorescent protein tags, Presley *et al.* visualized the binding and dissociation of these proteins *in vivo* by monitoring fluorescence changes in response to selective photobleaching within the cell. For example, Presley *et al.* selectively photobleached coatomer bound to the Golgi membrane (panel 1, prebleaching; Golgi region is outlined in panel 2) with a high intensity laser light. Recovery of fluorescence in the Golgi region — due to binding of unbleached tagged coatomer to the Golgi — was then monitored using images collected after photobleaching (panels 3, 4). Analysis of the fluorescence recovery process provides binding and dissociation kinetics.



The observations reported by Presley *et al.* challenge the accepted model of Arf1/coatomer dynamics. Presley *et al.* found that coatomer and Arf1 are continuously binding to and dissociating from the membrane in a stochastic fashion, even at temperatures as low as 4 °C, where vesicle budding would not occur. Thus, Arf1/coatomer binding is not directly coupled with vesicle budding as was generally assumed.

Additionally, while their results confirm that coatomer binding is in fact mediated by Arf1 binding and the dissociation of coatomer does depend on Arf1–GTP hydrolysis, the residence times of coatomer and Arf1 on the membrane are different. This contrasts with the generally accepted model where it was assumed that Arf1–GTP hydrolysis led directly to coatomer detachment. Although detachment requires Arf1–GTP hydrolysis, coatomer does not immediately detach upon hydrolysis. This allows coatomer to function in the recruitment of cargo proteins into kinetically stable membrane transport domains that last longer than the residence time of individual coatomer complexes on membranes.

The work of Presley *et al.* offers a first glimpse into the movement of Arf1 and coatomer within living cells and highlights (literally) the utility of fluorescent protein tags in studies of protein dynamics *in vivo*.

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