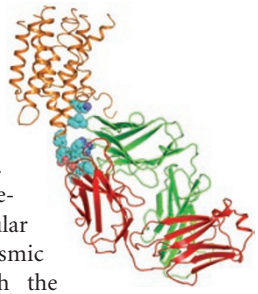
A fresh face for GPCRs



G-protein–coupled receptors (GPCRs) represent the largest family of membrane proteins and participate in a variety of important signaling events. GPCRs each have seven transmembrane-spanning helices (TMs), an extracellular N-terminal domain and a cytoplasmic C-terminal domain that interacts with the G-protein binding partner(s). There have been few structural studies of GPCRs, as they are inherently unstable and difficult to produce recombinantly. Much of what is known about GPCR structure comes from crystallographic studies of bovine rhodopsin, a relatively robust molecule. Now, a structure of the human β_2 adrenoreceptor (β_2AR) in complex with an inverse agonist at 3.4-Å resolution, from the Weis and Kobilka groups, offers another structural model for consideration. While the structure of the TMs is overall very similar to rhodopsin, it provides new insights into the structural basis of GPCR basal activity. In contrast to rhodopsin, β_2AR has basal activity, even in the presence of the inverse agonist included in crystallization. This basal activity may be reflected structurally by the larger distances between the cytoplasmic ends of TM3 and TM6 compared to rhodopsin, causing loss of key ionic interactions known to lock rhodopsin in an inactive conformation and to modulate unliganded β_2AR activity. However, the presence of packing interactions of a leucine residue in TM6, also known to attenuate basal receptor activity, suggests that the receptor may have multiple basal activity states. The techniques used to obtain the β_2AR structure may prove useful for other researchers examining this important class of proteins. (*Nature* advance online publication 21 October 2007, doi:10.1038/nature06325) *MM*

Ribosome recycling

When the ribosome active site encounters a termination signal and halts translation, the complex does not immediately dissolve. Instead, a post-termination complex (post-TC) is formed, in which the messenger RNA and a deacylated transfer RNA sitting in the P site remain bound within the ribosome. Bacteria dissociate the post-TC using three factors: IF3, an initiation factor, EF-G, an elongation factor, and RRF, a ribosome-recycling factor. EF-G and RRF break up the complex into a free 50S subunit and a 30S subunit still bound to mRNA and P-site tRNA. IF3 then facilitates removal of the tRNA, which allows the mRNA to release the 30S subunit. The situation in eukaryotes has been less clear, however, because they lack an RRF homolog and the interaction of their release factors with the ribosome is different. Pestova and colleagues developed an *in vitro* model system to test the hypothesis that post-TC recycling in eukaryotes involves only initiation factors. They determined that four initiation factors—eIF1, eIF1A, eIF3 and eIF3j—are required for recycling; there is no dedicated recycling factor. Of these, eIF3 is the central factor that can, by itself, dissociate the ribosome subunits. The authors speculate that binding of eIF3 to the 40S ribosome changes the ribosome's conformation so that eIF1 and eIF1A can interfere with 40S-60S subunit interactions. eIF1 also has a role in the release of deacylated tRNA from the P site of the recycled 40S subunit, after which eIF3j promotes mRNA dissociation. Thus, although different factors are used in prokaryotes and eukaryotes, the overall process of dissociating the ribosome subunits first, followed by the tRNA and then the mRNA, is conserved. (*Cell* **131**, 286–299, 2007) *AKE*

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ATR as a first responder

Genome integrity is monitored by DNA damage checkpoint pathways that delay or arrest the cell cycle in response to DNA damage. DNA damage checkpoints can cause G1/S arrest to prevent replication of damaged DNA or G2/M arrest to prevent segregation of damaged chromosomes during mitosis. DNA replication itself is also tightly regulated, and inactivation of Geminin, an inhibitor of origin licensing, leads to re-replication and triggers a G2/M checkpoint. Re-replication produces both single- and double-strand DNA breaks, and activates both ATR/Chk1 and ATM/Chk2 pathways. Lin and Dutta examined the relative importance of these two pathways in a human colorectal cancer cell line and found that Rad9 and Rad17 proteins are required for the accumulation of re-replicated cells and G2/M activation after Geminin depletion. Rad9 is part of the Rad9–Rad1–Hus1 (9-1-1) complex, and Rad17 is a checkpoint protein homologous to replication factor C 1 (RFc1) that associates with other RFC subunits to form a clamp loader. In contrast, Mre11 and ATM are not required for re-replication. Moreover, the authors found that Chk1 is activated around the time of G2/M arrest but before the accumulation of re-replicated cells, whereas Chk2 activation occurs relatively late. On the basis of these results, they suggest a hierarchical checkpoint-activation pathway in which Geminin depletion leads to the generation of single-stranded DNA in re-replicated cells and induction of the G2/M checkpoint mediated by ATR. At later stages of re-replication, fork collapse and replication across single-strand nicks can create double-strand DNA breaks that activate ATM/Chk2 and p53. In this way, the cell would have the chance to repair relatively minor damage caused by re-replication during G2 phase, but could still activate p53 and apoptosis if re-replication persisted. (*J. Biol. Chem.* **282**, 30357–30362, 2007) *BK*

Hijacking Rab1

Legionella pneumophila offers a remarkable example of how intracellular pathogens exploit the functions of host cells. Once internalized into a phagosome, *L. pneumophila* remodels this compartment into an endoplasmic reticulum (ER)-derived vacuole, called LCV, that is favorable for its replication. LCV maturation is accomplished by the recruitment of cellular Rab1, a GTPase involved in the transport of ER vesicles. Activation of a Rab GTPase requires the displacement of its GDP/GTP dissociation inhibitor (GDI) by a GDI-displacement factor (GDF), followed by Rab recruitment to the membrane and activation by GDP/GTP-exchange factors (GEFs). Finally, Rab-GTP is converted into Rab-GDP at the membrane by the action of a GTPase-activating protein (GAP). To create the LCV, Rab1 is recruited by a bacterial GEF called DrrA/SidM, but how Rab1 is released from its GDI remained unclear. Now, two independent groups have characterized the dual function of DrrA/SidM: besides its GEF activity, this bacterial protein also acts as a GDF to release Rab1 from its cognate GDI. In addition, Machner and Isberg report that a second bacterial Rab-interacting protein, LidA, helps accumulate activated Rab1 on LCVs. Roy and colleagues uncover a novel Rab1 interaction with bacterial factors: LepB stimulates GTP hydrolysis by Rab1 and Rab1's consequent inactivation when LCV maturation is complete, thus functioning as a GAP. These results illustrate the exquisite mechanisms evolved by pathogens to manipulate cellular processes for their own benefit. (*Science* published online 18 October 2007, doi: 10.1126/science.1149121; and *Nature* advance online publication 21 October 2007, doi:10.1038/nature06336) *IC*