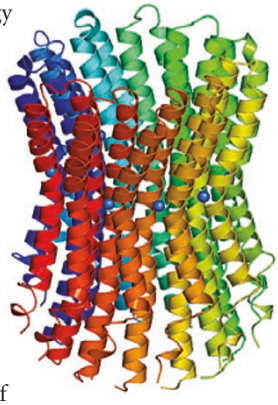
Pumped up



Almost all cellular processes require energy input. Inside cells, energy can be stored in various forms, including the high energy bond in ATP and the electrochemical potential created by an ion concentration gradient across a membrane. Two structurally related protein complexes can catalyze the conversion between these two energy sources: the F-type ATPases that transform the electrochemical potential into ATP and the V-type ATPases that consume ATP to generate an ion concentration gradient. Both F-type and V-type ATPases consist of two coupled 'motor' components: a soluble F_1 (or V_1) complex with ATP hydrolysis activity and a membrane-bound F_o (or V_o) complex that transports ions across the membrane. These two motors are connected by a central 'shaft' and a peripheral 'stalk', and the relative rotation of the rotors with respect to the stator assemblies is important for their activities. The mechanism of ATP hydrolysis (and synthesis) by the F_1 motor has been examined by various experimental approaches, but how ions are transported by F_o or V_o has not been clear due to a lack of high-resolution structures. Walker, Leslie and colleagues have now determined the crystal structure of the rotor ring complex in the V_o motor of a bacterial V-type Na^+-ATPase. In an independent study, Dimroth, Diederichs and colleagues have solved the structure of the corresponding ring from the F_o motor of a bacterial F-type Na^+-ATPase. The V_o ring contains 10 identical subunits, each with four slightly curved α-helices. Two of the four helices from each subunit line the inside wall of the ring. In comparison, the F_o ring contains 11 identical subunits. Each subunit contains two bent α-helices, and the overall shape of the complex resembles that of an hourglass. The N-terminal helix from each subunit lines the inside wall of the ring. Thus, although the F_o and the V_o rings are of similar height, the V_o ring has a much larger diameter than the F_o ring. In both structures, bound Na^+ ions are found on the outside of the rings, in a locked conformation coordinated by conserved residues. The Na^+ ions are most likely near the center of the lipid bilayer, with no obvious access channels to either side of the membrane. These structures thus do not reveal the ion-conducting path of the rotors. Nevertheless, the structures could facilitate the design of new experiments to provide details about the ion transport mechanism. (*Science* **308**, 654–659 and 659–662, 2005) *HPF*

Tuning EGF out

Epidermal growth factor (EGF) promotes cell growth and activation by binding to and activating the EGF receptor (EGFR). Inside the cell, the activated EGFR signal is transmitted to Grb2, which recruits the guanine nucleotide exchange factor Sos-1 to the plasma membrane. This leads to the sequential activation of the Ras G-protein, Raf kinases, and MAP or ERK kinases (MEKs), which leads to gene expression via the ERK signaling pathway. The cyclin-dependent kinase Cdc2 promotes cell cycle progression through G2 and M phases. During mitosis, transcription and translation, as well as other cellular processes that can use up much–needed energy stores, are inhibited by Cdc2. In addition, mitotic cells are less responsive to EGFR tyrosine kinase activation. Cdc2's kinase activity has been implicated in inhibiting EGFR and ERK activity in mitotic cells, but it is not known if Cdc2 acts on the components that link EGFR to the ERK pathway. To address this question, Dangi and Shapiro examined ERK activation by EGF stimulation in mitotic cells. They show that during mitosis Cdc2 interacts with Sos-1, Grb2, and Raf-1, and that its kinase activity is partly responsible for hyperphosphorylation of Raf-1 and Sos-1, the latter of which may impair Grb2-Sos-1 interactions. EGF-stimulated ERK activity during mitosis is inhibited when cells are transfected with constitutively active Ras or Raf-1, but not MEK-1. Cdc2 inhibition is able to restore EGF-, Ras- and Raf-1-mediated ERK activation. The data suggest that Cdc2 is able to inhibit EGFR-mediated ERK activation upstream of the MEKs, indicating that other processes are involved in regulating ERK activity in G2 and M phase progression. (*J. Biol. Chem.* 11 May 2005, doi: 10.1074/jbc.M414079200) *MM*

RNA on acid

Proteins use several mechanisms to catalyze reactions. For example, during a proteolysis reaction catalyzed through the general acid/base mechanism a proton is transferred between an amino acid side chain, such as histidine, and a substrate. Alternatively, proteins can use a metal ion to activate a water molecule or side chain for reaction with the substrate. Until recently, RNA molecules, which can also catalyze similar reactions as proteins, were thought to utilize only metalloenzyme chemistry. Das and Piccirilli now show that RNA nucleobases can function as general acids or bases. They combined mutagenesis with chemical perturbation of leaving groups to investigate the role of an active site cytosine in the hepatitis delta virus (HDV) ribozyme self-cleavage reaction. The authors mutated the active site cytosine (C76) to a uracil thereby eliminating the N3 nitrogen that could donate a proton in the reaction. This C76U mutation severely affected the phosphodiester cleavage reaction. In contrast, the authors show that an RNA substrate-containing a 5′-bridging phosphorothiolate linkage in which a sulfur atom replaces the 5′ oxygen in the phosphodiester backbone—a modification that generates a hyperactivated leaving group—suppressed the effects of the C67U mutation. These data provide evidence that C76 is involved in activating the leaving group. To determine if C76 was acting as a proton source during the reaction, the authors constructed a mutant ribozyme containing a cytosine analog that alters the acidity at C76. They use this mutant to show that the reaction slows down with increasing pH because there is less protonated cytosine available. These and other data are consistent with C76 functioning as a general acid and donating a proton to the leaving group during HDV catalysis. These results expand the repertoire of RNA catalytic mechanisms and show that RNA nucleobases can function in much the same way as histidine residues in protein enzymes. (*Nat. Chem. Biol.* advance online publication, 3 May 2005 doi: 10.1038/nchembio703) *EJ*

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