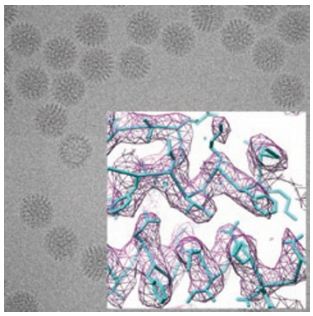
Just a closer look at thee



'Single-particle reconstruction' is an image processing method that can be used to convert the two-dimensional images obtained from electron cryomicroscopy (cryoEM) of a biological macromolecule into a three-dimensional structural model. Although this technique is powerful—it has been used to elucidate the three-dimensional structures of virus particles, large protein complexes and other biological macromolecules—it is usually not possible to use this method to determine the detailed structure of a biomolecule at atomic resolution. Grigorieff, Harrison and colleagues have recently demonstrated that cryoEM and single-particle reconstruction can be used to obtain striking EM maps of viral protein 6 (VP6) from the rotavirus inner capsid particle (the 'double-layer particle'). The authors generated a three-dimensional reconstruction of VP6 from 8,400 images of rotavirus particles using the computer program FREALIGN. The positions and conformations of many amino acid side chains were clearly visible in the EM density maps, which were nearly indistinguishable from 3.8-Å resolution electron density maps of VP6 obtained using X-ray crystallography. Although the icosahedral symmetry of the viral particle had a key role in allowing high-quality EM maps to be obtained (as this essentially increased the amount of data by 60-fold), advances involving sample preparation and image processing may enable structural biologists to obtain atomic-resolution models of less symmetric biological macromolecules using cryoEM in the near future. (*Proc. Natl. Acad. Sci. USA* **105**, 1867–1872, 2008) *JMF*

Regulating tail addition

Eukaryotic mRNAs are stabilized against degradation by the addition of a stretch of adenosine residues (the poly(A) tail) to the processed 3′ end. This process is catalyzed by a specialized nucleotidyl transferase known as poly(A) polymerase (PAP). PAP is regulated by alternative splicing, acetylation and phosphorylation. In this work, Manley and colleagues define an additional regulatory mechanism, mediated by addition of the small modifier SUMO. An interaction between PAP and the SUMO E1 activating enzyme, Uba2, was known, but the significance was undetermined. Here the authors found that PAP showed a ladder of bands that reacted with antibody toward SUMO-2/3, which can form polymeric SUMO chains on target proteins. Unusually, most PAP undergoes modification, despite the protein's lack of a SUMOylation consensus sequence. Nevertheless, PAP interacts with Ubc9, the SUMO E2, and can be modified *in vitro*. Mapping revealed that there are potentially six sites of modification, although only two sites seem to have a strong functional effect. SUMOylation affects the nuclear localization of PAP, enhances its stability and inhibits its polyadenylation activity. Given these varied effects on PAP, further experiments are required to provide a cohesive model explaining how SUMOylation, particularly in relation to other post-translational modifications, regulates PAP activity under different contexts and how this further affects gene expression. (*Genes Dev.* **22**, 499–511, 2008) *AKE*

Written by Inês Chen, Angela K Eggleston, Joshua M Finkelstein & Michelle Montoya

It takes Rb to make RBCs

Red blood cells (RBCs) contain mainly hemoglobin to transport oxygen from the lungs to all tissues in vertebrates. RBCs are produced through a process called erythropoiesis, comprising several differentiation and cell-division steps followed by the cells' exit from the cell cycle as erythroblasts; these cells lose their nucleus and then organelles to finally become mature RBCs. The process is well known from a cytological point of view, and it is now being unraveled at a molecular level. The retinoblastoma protein (Rb) controls cell-cycle progression from G1 to S phase, and its role in erythropoiesis has been controversial, with conflicting data from *Rb*-null mice (which are embryonic lethal), chimeric and transplant models, and *in vitro* culture approaches. Orkin and colleagues have now produced mice with the Rb gene deleted specifically in the erythroid cell lineage, allowing them to demonstrate the direct role of Rb in erythropoiesis *in vivo*. The animals are moderately anemic, and, by examining their erythroid-lineage cells, the specific step blocked by the lack of Rb was identified as a post-mitotic transition from early to late erythroblasts. Transcriptional profiling of these cells revealed the upregulation of S-phase genes and the downregulation of mitochondrial genes. Biosynthesis of heme occurs in the mitochondria, and high levels of ATP are important to make globin, the protein component of hemoglobin, and other biosynthetic processes. Thus, Rb controls two cellular events in the terminal maturation of erythroblasts: cell-cycle exit and mitochondrial biogenesis. The Rb pathway has been well studied, and the authors propose a model for how Rb coordinates these events; it will be exciting to see future work exploring these molecular interactions in erythropoiesis and other differentiation processes. (*Genes Dev.* **22**, 463–475, 2008) *IC*

Signaling distinctions

The transforming growth factor-β (TGF-β) superfamily transmits extracellular signals across the cell membrane by assembling two pairs of type I and II receptors, whose extracellular ligand binding domains have the same fold. One branch of this superfamily is represented by the bone morphogenetic proteins (BMPs), whose receptors have differing affinities for ligands, do not interact with each other and produce a graded signal output that may in part be caused by avidity due to membrane localization. A second major branch includes the prototypical TGF-β, which binds with high affinity to its type II receptor (TβRII) to cooperatively recruit the low-affinity binding type I receptor partner (TβRI) for transphosphorylation and signal transduction. Previous structural work had shown that TGF-βs and BMPs interact with different surfaces on their type II receptors, but interactions with their type I receptors were assumed to be similar. Now, the structure of a ternary complex that includes a TGF-β3 dimer and the extracellular domains of two TβRIs and two TβRIIs, determined by Groppe and colleagues, indicate otherwise. The cooperative recruitment of TβRI is revealed to use receptor-receptor interactions at a preformed TGF-β–TβRII interface that makes use of a loop extension present in TβRI and requires ordering of the N terminus of TβRII. *In vitro* and cellular assays confirmed the importance of these interactions for TGF-β signaling. Comparison with the binary complex of BMP2–BMPRIA shows that the ligand–type I receptor interactions are different, with TβRI establishing more contacts with a monomer of the ligand dimer than BMPRIA. The authors suggest that the cooperative and specific interaction between TGF-β ligand and receptor pairs is required for their all-or-none type signaling response, in contrast to the dose-dependent activity of their BMP relatives. (*Mol. Cell* **29**, 157–168, 2008) *MM*