The major impact of these new studies is that they further question the classical hierarchical model of Polycomb recruitment and provide data suggesting that the order of recruitment could even be reversed. The observation that H2Aub1 alone is able to trigger PRC2 recruitment leads Blackledge *et al.*[2] and Cooper *et al.*[3] to propose that H2Aub1 itself could recruit PRC2 (**Fig. 1b**). However, the existence of a cofactor or a subunit domain that mediates PRC2 binding to H2Aub1 is speculative at present, and the question of whether recruitment of PRC2 upon H2A monoubiquitination is direct or indirect remains to be addressed. This issue is tightly linked to the more general question of the mechanisms underlying timely and target-specific *de novo* recruitment of PcG complexes, which remain to be determined (**Fig. 2**). For instance, the absence of transcription might be sensed by PcG complexes or their recruiters and may be sufficient to trigger their independent but simultaneous recruitment to CGIs (**Fig. 2**; scenario **a**-**b**-**c**-**e**). Alternatively, targeting repressive H2Aub1 could be sufficient to induce transcription silencing, which in turn could trigger PRC2 recruitment (**Fig. 2**; scenario **a**-**c**-**e**). Importantly, knockout of the catalytic subunits of PRC1 has no effect on bulk H3K27me3 levels in either *Drosophila* imaginal discs of third-instar larvae or mouse ESCs[19,20], indicating that PRC1 is not involved in the maintenance of H3K27me3. In contrast, time-course analysis upon induction of Ring1A and Ring1B deletion in mouse ESCs shows that PRC2 binding decreases at target genes simultaneously with recruitment of RNA polymerase II[19]. Therefore, PRC2 displacement as a result of inactivation of PRC1 complexes could be due to transcriptional activation rather than the loss of H2Aub1. Unraveling the precise recruitment paths actually used by the cell, under which circumstances and in order to achieve which biological outcomes, remains one of the major challenges to understanding the role of the PcG proteins in development and disease.

COMPETING FINANCIAL INTERESTS
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Channeling your inner energy

José D Faraldo-Gómez

ATP is continuously synthesized inside mitochondria and exported to the cytoplasm via transporter and channel proteins residing in the inner and outer mitochondrial membranes, respectively. In this issue of *Nature Structural & Molecular Biology*, a new crystal structure of the mitochondrial channel protein VDAC-1 provides the basis for a detailed simulation study that unravels the mechanism by which ATP diffuses across the outer mitochondrial membrane at a fast rate.

The free energy derived from the hydrolysis of ATP sustains, directly or indirectly, much of the activity in cells. ATP is also used in intracellular and extracellular signaling, and it provides one of the building blocks of DNA and RNA. It has been estimated that an active cell consumes as many as several million ATP molecules per second; each human being, with about 100 trillion cells, therefore turns over roughly one body-weight equivalent in ATP every day. In eukaryotic organisms, most of this ATP is produced inside mitochondria through a series of metabolic reactions collectively referred to as aerobic respiration. ATP generation is in fact akin to a recycling process, in that the products of the hydrolysis reaction, ADP and inorganic phosphate (P_i), are recovered by mitochondria and are forcibly rejoined by an enzyme known as ATP synthase. Thus, cellular bioenergetics depends on the continuous export and import of ATP and its hydrolysis byproducts across the mitochondrial envelope.

The voltage-dependent anion channel (VDAC) facilitates the permeation of ATP through the outer mitochondrial membrane[1,2]. VDAC also channels ions and other metabolites[3], including ADP and P_i, and in addition has an important role in the mitochondrial apoptotic pathway[4], which makes it a potential chemotherapy target against cancer[5]. When studied *in vitro*, VDAC exhibits an intriguing ability to adopt several conformational states in response to applied transmembrane voltages, each with distinct conductance and selectivity properties[6,7]. The so-called high-conductance state that permits flux of ATP and ADP, among other anionic species, is the predominant form under normal physiological conditions and accordingly is observed for small or absent transmembrane voltages.

Although it is universally accepted that most of the VDAC polypeptide folds itself into a transmembrane β-barrel, the precise architecture of the high-conductance form remains a controversial matter. Numerous and often-incongruent hypothetical models have been put forward over the past three decades, on the basis of diverse biochemical data as well as bioinformatic and theoretical analyses[8]. The debate seemed to come to an end in 2008, when three different groups reported high-resolution structures of the VDAC-1 isoform from humans and mice, obtained independently by NMR[9], X-ray crystallography[10] or a combination of both[11]. All three groups followed a strategy that had

José D. Faraldo-Gómez is at the Theoretical Molecular Biophysics Section, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland, USA.
e-mail: jose.faraldo@nih.gov

proven successful in earlier structural studies of membrane proteins, namely to heterologously express the protein in a host organism (in this case *Escherichia coli*) and to refold it from inclusion bodies after denaturation. Otherwise, the specifics of the biochemical procedures in these studies differed, as did the technique used for structural determination. Nevertheless, the three VDAC-1 structures are largely identical and consist of an unprecedented 19-stranded β-barrel and an intriguing N-terminal α-helix that protrudes into the channel lumen and is packed against the barrel wall. Although atypical, the dimensions of this β-barrel are compatible with earlier electron- and atomic-force-microscopy studies of VDAC in mitochondrial membrane fragments[12]. Notwithstanding, the biological significance of the VDAC-1 structures has been questioned[13,14]. It has been argued that the 19-stranded barrel is at odds with various biochemical and biophysical experiments and that the published NMR and crystal studies capture a non-native conformational state favored during the *in vitro* refolding of the protein. Not surprisingly, the authors behind the discovery of the VDAC-1 structures have disputed this view, pointing out, among other arguments, several examples of bacterial outer-membrane β-barrel proteins whose refolded structures have been shown to be largely identical to those of the native proteins[12].

In this issue, Choudhary *et al.*[15] present a crystal structure of mouse VDAC-1 that is highly similar to that previously published[10], consistently with its being obtained in an analogous fashion, i.e., from refolded protein crystalized in a lipidic medium. In the current study, however, the protein crystals were soaked in a solution containing large amounts of ATP and magnesium, a technique often used to identify ion- and substrate-binding sites in membrane proteins amenable to crystallization. The authors were thus able to detect what appears to be a weak-affinity binding site for ATP within the channel, indicating that the crystallized form of mVDAC-1 is indeed permeable to ATP, as would be expected from the physiological, high-conductance form of the protein. ATP is not as well resolved as in other ATP-bound structures of comparable resolution, probably because the electron density reflects a variety of configurations of the molecule; this might also explain why a coordinating Mg^{2+} ion could not be detected.

Choudhary *et al.*[15] provide additional evidence in support of the notion that the crystal structure of VDAC-1 represents a physiologically relevant state, through a sophisticated computational analysis of its permeability to ions and ATP. In a nutshell, the authors use atomistic molecular dynamics simulations of the protein embedded in a phospholipid bilayer and succeed in quantifying the permeation rates for K^+, Cl^- and ATP, finding the results to be in good agreement with values measured for the physiological form of VDAC. To bridge this gap between structure and function is, however, much easier said than done, particularly for ATP, and therein lies what is most remarkable about this study.

For most systems of interest, the timescale that can be probed during a single all-atom molecular dynamics simulation is typically shorter than that of the nontrivial biological process that motivates the simulation study. This paradox is somewhat perplexing for many structural biologists and biophysicists and may appear to be a valid argument for discounting molecular simulation methods from the toolbox of modern biological research. However, as Choudhary *et al.*[15] are able to illustrate, the inherent properties of a molecular system, such as the conductance of a channel or even the conformational free-energy landscape of a protein[16,17], can be derived from simulated molecular dynamics trajectories spanning a limited timescale, if the calculations are designed and analyzed in the context of rigorous theoretical treatments rooted in statistical thermodynamics. These approaches, however, come at a large computational cost; therefore, serious simulation studies typically require many months, if not years, of sustained effort—not unlike experimental research.

Choudhary *et al.*[15] use an advanced simulation technique known as Markov-state modeling, in which the process under study is seen as a series of fast elementary transitions across a network of interconnected, intermediate states. To begin with, a diverse set of putative configurations of the molecular system is obtained from a strongly biased exploratory approach that allows for extensive coverage of configurational space at an affordable computational cost. This initial distribution of states, which is naturally very different from that at equilibrium, provides a starting point for hundreds of conventional, unbiased molecular dynamics simulations, each of which need not be longer than ~100 ns, and which sample the configurational space in the locality of each of the starting points. The underlying free-energy landscape of the molecular system will lead these simulations to explore primarily a finite set of well-defined configurations and to exchange between them. Through a global analysis of the collected simulation data, the most-probable statistically independent states (or Markovian states) may be identified, and the frequency of interconversion between them can be quantified. Further analysis of the resulting transition probability matrix reveals the most prominent pathways along which the system evolves in time as well as the characteristic timescale of that evolution.

In a remarkable *tour de force*, Choudhary *et al.*[15] collect 40 μs of sampling time from roughly 500 independent simulations and identify over 200 distinct ATP configurations within and proximal to VDAC. The resulting Markov-state model not only identifies the configurations observed in the crystal structure as being among the most probable but also predicts a characteristic permeation rate that is in excellent quantitative agreement with values derived from electrophysiological measurements. Interestingly, this analysis reveals that ATP permeation proceeds via a multiplicity of pathways, along the way forming alternate electrostatic-interaction patterns with basic side chains lining the pore lumen.

As yet, however, the controversy around VDAC is not quite resolved. Although all published theoretical studies of the NMR and crystal structures of VDAC-1 report conducting properties consistent with those expected from the native protein[18–22], it is not completely out of the question that alternative architectures[14,23] would be consistent as well. We can only hope that additional experimental and computational work will eventually lead to a consensus.

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PAN-orama: three convergent views of a eukaryotic deadenylase

Sophie Martin & Jeff Coller

Post-transcriptional mRNA regulation is often attained by lengthening or shortening the 3′ poly(A) tail of a transcript. Eukaryotic mRNAs show a spectrum of deadenylation rates, thus allowing intricate control of gene expression, but the mechanisms that determine such rates are unclear. Three new studies highlight the structural and biochemical features of a key enzyme in removing poly(A) tails, the PAN2–PAN3 complex, providing clues to how different mRNA deadenylation rates can be achieved.

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Regulating an mRNA's fate is arguably as important as regulating its synthesis. Indeed, mRNA post-transcriptional regulation is of paramount importance in certain contexts (for example, the embryo or the brain)[1]. Importantly, mRNAs can be turned 'on' or 'off' in response to biological cues, and the switch is often the poly(A) tail, a ubiquitous modification added to nearly all eukaryotic messages[2] (**Fig. 1**). The tail stabilizes and facilitates translation of mRNAs, and the switch between active and inactive states is controlled by adding or removing the tail, i.e., by adenylation or deadenylation. Understanding how adenylation and deadenylation are controlled is central to understanding how gene expression is finely tuned. Three new studies[3–5], including two in this issue of *Nature Structural & Molecular Biology*, provide a fresh new perspective on an enzyme that helps promote poly(A)-tail removal.

Although much is known about polyadenylation, the regulation of deadenylation is still somewhat mysterious. The activating effects of the poly(A) tail are accomplished through the highly conserved poly(A)-binding protein (PAB)[6,7]. In budding yeast, an enzyme that removes bulk poly(A) tails was described in the early 1990s, when it was shown that extracts deficient in PAB are incapable of deadenylation[8,9]. Adding recombinant PAB resulted in tail shortening, from 100 nt to ~50 nt. Thus, a PAB-dependent poly(A) nuclease (PAN) was sought and identified as being encoded by two genes, *PAN2* and *PAN3* (refs. 10,11). As in the original description, PAN2–PAN3 was capable of removing only the first poly(A) residues, suggesting that deadenylation was biphasic. The biphasic nature of deadenylation made sense, given that PAN is dependent on PAB; thus, PAN2–PAN3–dependent deadenylation would eventually stop once PAB was displaced (usually around 25 As). Enzyme complexes responsible for the second phase of deadenylation were later described (for example, the CCR4–NOT complex, which is inhibited by PAB)[12,13]. The PAN2–PAN3 and CCR4–NOT complexes are suggested to function sequentially, with PAN2–PAN3 acting first (displacing PAB), then CCR4–NOT[14,15].

Much attention has been given to the CCR4-NOT complex, but less is known about PAN2–PAN3. PAN2 is the catalytic subunit of the complex, but the role of PAN3 is unclear. The singular defining feature of the PAN2–PAN3 deadenylase is that its activity was initially shown to be dependent on PAB[10,11]. This dependency was the sole explanation for why PAN2–PAN3 performed only initial deadenylation and then stopped—the tail was too short to support PAB binding; therefore, PAN2–PAN3 was no longer active. Now three groups have solved crystal structures of the PAN2–PAN3 complex that, along with functional analyses, collectively provide information on the precise roles of PAN2 and PAN3 in deadenylation.

Strikingly, Schäfer *et al.*[4] and Wolf *et al.*[5] show that purified and reconstituted PAN2–PAN3 complexes have intrinsic, PAB-independent deadenylase activity *in vitro*. Moreover, even in PAB's absence, PAN2–PAN3 deadenylates with biphasic kinetics, with an initial fast step followed by a much slower second step. Although it is clear that PAB stimulates the rate of the reaction, it is the preservation of the biphasic nature of PAN2–PAN3 deadenylation that is most curious. The explanation emerges from the structural nuances of the PAN2–PAN3 complex observed in the three studies.

The C-terminal domain of PAN2 is characteristic of the DEDD superfamily of exoRNases and confers the RNase activity. The study by Jonas *et al.*[3] shows that PAN3 does not have nuclease activity *in vitro* or when tethered to an mRNA reporter *in vivo*. Although PAN2 has robust deadenylase activity, its activity is strictly dependent on PAN3 (unless tethered)[3]. Consistently with this, PAN2 is incapable of binding RNA, whereas PAN3 binds well to RNA and has poly(A) specificity[5]. Together,

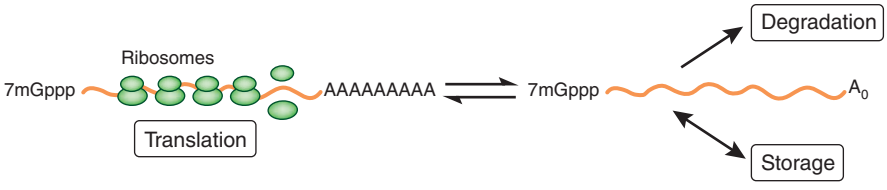

Figure 1 Poly(A)-tail length controls mRNA fate. The poly(A) tail stabilizes and facilitates translation, whereas deadenylation leads to either mRNA storage or decay. Tail length therefore permits a rapid mRNA on-off switch that allows cells to achieve fine regulation of gene expression. 7mGppp, 7-methylguanosine triphosphate.

Sophie Martin and Jeff Coller are at the Center for RNA Molecular Biology, Case Western Reserve University, Cleveland. Ohio, USA.
e-mail: jmc71@case.edu