POST-TRANSLATIONAL MODIFICATIONS

## *O*-GlcNAc in the dark

*Genes Dev.*, published online 10 February 2012, doi:10.1101/gad.182378.111;



The circadian rhythm is regulated by a set of clock transcription factors whose expression and activity depend on a number of interdependent, oscillating post-translational modifications. Kim *et al.* now provide evidence that the key clock transcription factor PERIOD (dPER) in *Drosophila melanogaster* is *O*-GlcNAcylated and that this modification affects the circadian clock in flies. The authors overexpressed dPER and the *O*-GlcNAc transferase (OGT) in a *Drosophila* cell line and observed that dPER is modified by *O*-GlcNAc. *In vivo* modulation of *O*-GlcNAcylation, by either knocking down or overexpressing OGT in neurons responsible for the *Drosophila* clock, resulted in flies with altered circadian activity. To validate *in vivo* that *O*-GlcNAcylated dPER is present and controls the circadian rhythm, the authors examined the *O*-GlcNAcylation of dPER during a 24-h cycle and observed an increase in the modification during the dark phase that could not be attributed to changes in OGT activity. Knockdown or overexpression of *ogt* in flies resulted in decreased or increased amounts of total dPER, respectively, which correlated with advanced or delayed nuclear localization timing. As a result, the authors concluded that *O*-GlcNAcylation of dPER occurs shortly after its synthesis and leads to increased protein stability and delayed nuclear entry via cytoplasmic retention. These results highlight a new role for *O*-GlcNAcylation in pacing the circadian rhythm. *AC*

Corrected after print 20 June 2012.

CHEMICAL PROBES

## PARP family portraits

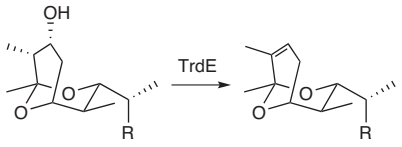
*Nat. Biotechnol.*, published online 19 February 2012, doi:10.1038/nbt.2121

Human poly-ADP-ribose polymerases (PARPs) share a common catalytic domain that facilitates the transfer of ADP-ribose. Perhaps the best-studied PARP family member is PARP1, which participates in the DNA damage response. On the basis of observations suggesting that blocking PARP1 sensitizes certain cancer cells to drugs or radiation, PARP1 inhibitors are currently being tested in clinical trials. The biological function of many of the remaining human PARP enzymes has not been well characterized, in part because of a lack of selective chemical probes. Thus, Wahlberg *et al.* set out to determine the specificity of known and potential PARP inhibitors. The team looked at the binding of 185 compounds, including clinical PARP inhibitors, to purified catalytic domains from 13 of the 17 human PARPs. One subset of compounds was relatively nonselective, binding up to 10 of the 13 PARPs tested. A second subset bound preferentially to PARP1, PARP2, PARP3 and PARP4. A third subset was selective for the tankyrases, PARPs initially described as telomerase regulators. Very few of the compounds tested, and none of the clinical compounds, were highly selective for a single PARP. Cocrystal structures identified distinct binding modes for nonselective, PARP1–PARP4-selective and tankyrase-selective compounds. These biochemical and structural insights may enable the development of highly selective tool compounds, including those for relatively uncharacterized family members such as PARP10 and PARP14 that catalyze mono-ADP-ribosylation rather than the prototypical poly-ADP-ribosylation. *JK*

BIOSYNTHESIS

## Hidden by homology

*J. Am. Chem. Soc.* **134**, 2844–2847 (2012)




The tirandamycins are biosynthesized by hybrid PKS-NRPS machinery, with several tailoring steps occurring after their release from the terminal carrier protein. TrdE is part of the tirandamycin cluster and shows high identity to glycoside hydrolase family 16, including the catalytic EXDXXE motif. However, tirandamycin does not contain any sugars, raising questions as to the true role of this enzyme. Mo *et al.* now demonstrate that TrdE provides a new biosynthetic mechanism for double-bond formation. Deletion of *trdE* generated an intermediate with a pendant hydroxyl group on the bicyclic ring, suggesting TrdE might remove this group prior to further tailoring. *In vitro* testing of TrdE confirmed this function, showing conversion of the hydroxylated compound—which the authors noted does structurally resemble a glycoside hydrolase substrate—into a dehydrated analog. To determine whether the glycoside hydrolase motif is relevant for catalysis, the authors mutated the conserved residues as well as four histidines on the basis of the known requirement for a histidine in other dehydratases. Only two of these mutants retained activity, in support of a typical glycoside hydrolase mechanism. Interestingly, mutations to three of the four histidines, close to each other in the primary sequence, caused substantial changes to the protein conformation, perhaps suggesting that a metal may be involved in stabilizing TrdE's structure. This functional reassignment may have relevance to other biosynthetic pathways with uncharacterized enzymes. *CG*

ALLOSTERY

## R for nonredundant

*Science* **335**, 712–716 (2012)

PKA is a cAMP-dependent kinase composed of two regulatory subunits (R) and two catalytic subunits (C) in its inactive holoenzyme state. Upon cAMP binding to the R subunits, the active C subunits are released. There are two classes of R subunits (RI and RII), each having two functionally nonredundant isoforms (α and β). The mechanism of PKA activation by cAMP and the reason the R subunits are nonredundant are not clear. Zhang *et al.* now report the crystal structure of the full-length RIIβ-C holoenzyme, stabilized by a mutation that abrogates cAMP binding, revealing a tetramer composed of two R-C heterodimers in which two residues that are known to interact with cAMP are kept from the cAMP binding site by a holoenzyme-specific salt bridge. The activation constant for the RIIβ tetramer by cAMP was higher than that for other holoenzymes, possibly owing to extensive interaction between the RIIβ-C heterodimers. By soaking Mg-ATP into their holoenzyme crystals, the authors were able to solve the structure of the enzyme—with reaction products Mg-ADP and a phosphorylated RIIβ subunit—which was similar to the holoenzyme structure, indicating that the holoenzyme contains a well-formed active site in the C subunit and that allosteric activation may be required to release the reaction products. This first structure of a full-length RIIβ-C holoenzyme provides insight into allosteric activation by cAMP and suggests that differential contacts among heterodimers may underlie nonredundancy of R subunits. *AD*

ERRATUM

# Research Highlights

*Nat. Chem. Biol.* **8**, 320 (2012); published online 16 Mar 2012; corrected after print 20 June 2012

In the version of this article initially published, the doi for the article referred to in the highlight entitled "O-GlcNAc in the dark" is incorrect. The correct doi is 10.1101/gad.182378.111. The errors have been corrected in the HTML and PDF versions of the article.