conformation in the active site (Hsiao et al., 2011). Because only single-stranded RNA was used for this study (Romano et al., 2014), it will be interesting to know if RNase AS can also degrade double-stranded RNA with 3′ overhangs and whether its activity will be blocked by double-stranded structures.

In prokaryotes, the poly(A) tail functions in the regulation of RNA stability and quality control (Mohanty and Kushner, 2011). Given that RNase AS and PARN [a poly(A)-specific 3′ exoribonuclease of the DEDDh subfamily; Wu et al., 2005] share a similar substrate preference, and the latter is a key deadenylase in eukaryotic mRNA turnover, RNase AS is likely involved in mRNA decay through shortening of the 3′ adenylate-containing mRNA. However, the transcripts targeted by RNase AS are not known. One of the future goals is to identify the genes responsible for the mycobacterial virulence whose stability is regulated by RNase AS through deadenylation. Identification of the physiological substrates of RNase AS and elucidation of the catalytic mechanism of this important RNase would greatly aid the drug design efforts toward more efficient TB therapy.

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Membrane Interaction and Functional Plasticity of Inositol Polyphosphate 5-Phosphatases

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In this issue of *Structure*, Trésaugues and colleagues determined the interaction of membrane-bound phosphoinositides with three clinically significant human inositol polyphosphate 5-phosphatases (I5Ps). A comparison to the structures determined with soluble substrates revealed differences in the binding mode and suggested how the I5Ps and apurinic endonuclease (APE1) activities evolved from the same metal-binding active center.

A complex family of membrane-bound inositol-lipid conjugates plays a central role in cell proliferation, synaptic vesicle recycling, and actin polymerization. Control of these pathways depends on a family of enzymes that control the phosphorylation of insoluble phosphoinositides (PtdIns), i.e., glycerolphospholipids bound to the 1-hydroxyl of *myo*-inositol. Specific kinases add phosphate groups at the 3, 4, and 5 hydroxyls of the inositol ring, leading to seven different isoforms. These, in turn, are removed by a family of phosphatases. In this issue of *Structure*, Trésaugues et al. (2014) show the binding site for three human phosphatases that remove the 5-phosphate from PtdIns with two or three phosphates bound to PtdIns. Mutations in the similar 5-phophatase active site of these enzymes are related to Lowes syndrome and Dent disease (5-phosphatase OCRL), characterized by renal failure, as well as defects in insulin signaling and obesity (5-phophatase SHIP2). They also determined the structure of INPP5B, whose similarity in substrate specificity to OCRL suggests it may have overlapping function. The details of these new structures could help in the design of treatments for renal syndromes. Inhibitors of SHIP2 might even be novel weight-loss medications, because mice deficient in SHIP2 were resistant to diet-induced obesity.

These structures are most remarkable for the details they can shed on how insoluble inositides bind, because the only structure of a 5-phosphatase catalytic domain previously reported is that of a complex of *Schizosaccharomyces pombe* synaptojanin (SPsynaptojanin) with a soluble ligand (Tsujishita et al., 2001). They are also important for the details they provide on a unifying mechanism for dephosphorylation of PtdIns with other types of phospho-transfer reactions.

The inositol position is different in the new structures from models of the

inositol binding site made using the SPsynaptojanin structure as a template. Although the active site clefts of the three human enzymes overlay well with that of SPynaptojanin, the orientation of the inositol in the membrane-bound substrate was dramatically different, by a rotation of about 100° from that seen in the binding of soluble substrates. The structures established two new lipid recognition motifs within the human enzymes that contribute to the recognition of the fatty acid part of the substrate. These membrane binding regions contain hydrophobic residues in most members of the 5-phosphatase family and could therefore be valuable targets for inhibitor design.

Unfortunately, the study did not reveal allosteric mechanisms that would correlate lipid binding with the catalytic activity at the P5 position. The inositol ring is not planar, and addition of phosphates at different positions alters its 3D pucker. Therefore, even small changes in its binding mode can be very important for designing specific inhibitors. The interactions of the phosphates in the inositol ring to the enzymes are mainly through positively charged Lys or Arg residues, a feature found in other inositol phosphate complexes. This is consistent with, for example, the relatively surface exposed activator site for inositol-6-phosphate (phytic acid) in the cysteine protease domains of *Vibrio cholera* or toxin B of *Clostridium difficile* (Pruitt et al., 2009; Lupardus et al., 2008). There, while each Pi is bound by several positively charged side chains, there are no direct contacts to the ring. This suggests that a flexible binding site may be necessary to allow for changes in configuration of the ring induced by phosphorylation changes.

Trésaugues et al. (2014) provide convincing evidence for a similar active center for the 5-inositol phosphatases and the apurinic base excision repair endonucleases that had previously been predicted by fold recognition methods, sequence conservation, local structural similarities (molegos), and mutagenesis studies (Whisstock et al., 2000; Schein et al., 2002). The authors compared the cleft where the scissile 5-P was observed in the (postcleavage) 5-phosphatase structures and the single Mg^{2+} ion site to structures of the substrate/metal/enzyme complexes determined for APE1. The position of conserved residues, including two aspartates, a glutamate, and especially the catalytic Asp/His pair (D308/H309 in APE1;D523/H524 in OCAL) are essentially superimposable across the five different proteins: the three human 5-phosphatases, SPsynaptojanin, and human APE1.

The conserved geometry suggests that the phosphatase and nuclease function of those distantly related enzymes could arise from an earlier phosphortransferase mechanism, perhaps from small sequence blocks that eventually became incorporated into larger, more specific enzymes (Schein et al., 2005; Mathura et al., 2003).

The question is: which came first, the phosphatase or the nuclease? A recent paper from Tainer and colleagues (Tsutakawa et al., 2013) suggests the latter. They were able to show that the active site of a repair enzyme with a different global fold, the bacterial endonuclease IV DNA (Nfo), was structurally related to that of APE1. Here, Zn^{2+} metal ions were able to assume the role of the conserved His residues in the I5P/APE1/Synaptojanin family. This example of a convergent evolution of the catalytic sites of the two endonucleases and the similarity of the 5-phosphatase and APE1 nuclease functional centers demonstrated in this paper show the strong structural requirement for a metal-based dephosphorylation and could mean that an analogous functional site might have even predated the enzymatic biological world. These recent observations on the structural and functional plasticity of catalytic active sites can fuel further research to explore potential pathways in the molecular evolution of functional sites, but they also pose a general question: what structural local similarities of functional sites are typically observed in distantly related enzymes?

The authors postulate a single metal transition state for the 5-phosphatases, based on their finding of only one magnesium ion in the OCRL active site. The question of exactly how many metals are required for cleavage and product release may seem esoteric, because reactions are usually run at concentrations that greatly exceed the molar equivalents of the enzyme or the substrate. It should be noted that the position of catalytic metal ions is difficult to ascertain in the phosphatases for several reasons. First, the presumed natural cofactor, Mg^{2+}, is a small ion that is hard to discriminate from water except in high resolution structures. Thus, many structures are done with larger metal ions, such as Mn^{2+}, or, in the case of APE1, even Yb^{3+} (a metal that enhances catalysis) or Pb^{2+} (which inhibits it). Second, the phosphate substrates themselves are Mg^{2+} ion magnets, meaning that the metal ions may be chelated by one residue of the protein (typically an aspartate) with waters or phosphate groups from the substrate. Finally, the metal ions may bind to other places on the protein or substrate, even, in some cases, stabilizing parts of the structure.

Previous phosphatase mechanisms have postulated that two or even three Mg^{2+} ions (Li et al., 2010; Gruninger et al., 2008) are necessary for activity. Trésaugues et al. (2014) point out that the single metal they observed is close to, but not identical to, that found in the single metal (Yb^{3+}) structure of APE1 (Mol et al., 2000) and nearly identical to the A position in the two-metal lead structure. APE1 is not strictly dependent on metal ions at all, with limited activity seen even in the presence of EDTA. We have suggested, based on molecular dynamics simulations of several different APE1 structures differing in the bound substrate and metal ion, that catalysis in APE1 could be done with one metal, whose pre- and postcleavage positions are different. The "moving metal," by accompanying the rotation of the phosphate during cleavage, could aid in rendering the reaction irreversible and facilitate transfer of the substrate to the next enzyme in the BER pathway (Oezguen et al., 2007, 2011; Schein et al., 2005). Because pre- and postcleavage positions for the metal ion may be quite different, details of the transition state of the I5Ps will thus depend on analysis of the enzymes bound to the uncleaved substrate as well as the products of the reaction.

In summary, these new co-crystal structures of human I5Ps, while confirming many details of other structures, have revealed some new and possibly controversial details of metal-ion-based cleavage of specific phosphate groups from the membrane-bound inositides. Because mutations in the three enzymes are directly linked to a variety of medical symptoms, these structures can have an important impact on the development of new drugs.

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Gas-Phase Structure of the *E. coli* OmpA Dimer

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In this issue of *Structure*, Marcoux and colleagues use gas-phase collisional cross section (CCS) measured by ion-mobility mass spectrometry to analyze the CCS of oligomeric states of *E. coli* outer membrane OmpA. CCS of the dimer supports a model of paired periplasmic C-terminal domains projecting away from the transmembrane porins.

The development of ion mobility mass spectrometry (IMS) has enabled the measurement of gas-phase collisional cross section (CCS) (Bohrer et al., 2008; Uetrecht et al., 2010). In this case, during their flight through the mass spectrometer, ions cross a specialized cell of inert drift gas under the influence of an electric field such that their shapes modulate their velocity, achieving a separation similar to the one obtained during electrophoresis. Exit of ions from the ion mobility cell is monitored by fast m/z measurement, allowing calibration with molecules of known shape/CCS and mass. IMS can thus be used to see if gas-phase structures are in agreement with the equivalent aqueous or crystalline species.

A major application for IMS is emerging in the study of native protein complexes largely because of the ability to measure CCS accurately, alongside mass, on time-of-flight instruments with extended m/z capabilities. The extra dimension of separation achieved with an ion-mobility separation prior to MS yields significant benefits in the simplification of spectra that might otherwise be uninterpretable. Importantly, complexes have been observed collapsing to states with smaller CCS or extending to states with larger CCS while maintaining their native oligomeric status (Ruotolo et al., 2005). Robinson's group pioneered the electrospray-ionization MS of native complexes and were the first to apply this technique to integral membrane proteins a decade ago when they demonstrated that detergent molecules retained after spraying micellar solutions of EmrE could be removed by gas-phase dissociation, with the dimeric protein retaining a bound cofactor through to the mass analyzer (Ilag et al., 2004). Considerable progress toward understanding native MS of membrane protein complexes has been made in the subsequent 10 years (Whitelegge, 2013; Laganowsky et al., 2013), and it is now possible to make confident measurements of membrane protein CCS for comparison with structural models.

The porin OmpA is the most abundant *Escherichia coli* outer membrane protein, but its functional significance is controversial because it is difficult to reconcile observed conductance measurements with an eight-stranded β-barrel formed by the N-terminal porin domain alone. Recently, in vivo crosslinking of *E. coli* cells with a specialized tri-functional reagent provided sound evidence for the presence of OmpA dimers in the outer membrane (Zheng et al., 2011). The distance constraints introduced by the reagent and the identity of the crosslinking sites localize the region responsible for dimerization to the "disordered" C-terminal domain. This crosslinking information provided the opportunity to model various potential dimer structures, and, more recently, to test these models via calculation of their respective CCSs for comparison to IMS measurements.

In this issue of *Structure*, Marcoux et al. (2014) found that native IMS of OmpA preparations revealed a mixture

