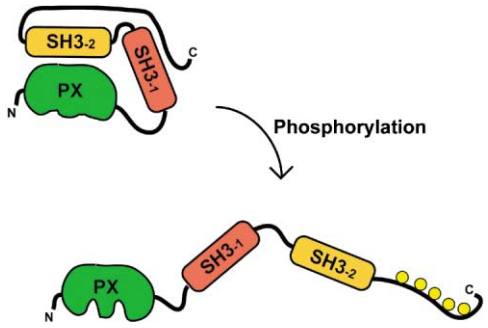

A Model for Activation of the p47phox Subunit of the NADPH Oxidase

In the resting state, the lipid binding ability of the p47phox PX domain (green) is masked via an intramolecular interaction between the PX domain and the second SH3 domain (yellow), while the C-terminal tail interacts with the first SH3 domain. Phosphorylation of serine and threonine residues in the C-terminal tail liberates the PX domain, which can now bind to lipids, while simultaneously allowing the SH3 domains to interact with other protein subunits of the NADPH oxidase complex.

space and time, and thereby prevent inadvertent damage to normal host tissues.

Curiously, one of the other proteins containing a PX domain with a presumptive PA binding pocket is phospholipase D itself, the very enzyme which cleaves phosphatidylcholine to produce PA in the first place! Furthermore, the N terminus of all PLD isoforms contains both a PX domain and an adjacent PH domain, suggesting that this protein's structure is yet another variation on the use of lipid binding domains to spatially integrate lipid kinase signaling events. Perhaps differential engagement of particular lipids by the PH domain and/or the two lipid binding pockets of the PX domain in PLD results in the selective activation, inhibition, or translocation to unique subcellular compartments.

The superfamily of lipid binding domains now includes PH domains, FYVE domains, ENTH domains, C2 domains, Tubby domains, and PX domains, and the list continues to grow. The unexpected finding by Karathanassis et al. that at least one member of a phospholipid binding family can simultaneously engage two lipid head groups suggests that additional surprises are likely to emerge as each of these domain families is investigated in detail. Whether the PX domain of p47phox is the only double header of the season remains to be seen.

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Recognition of Acetylated Proteins: Lessons from an Ancient Family of Enzymes

The structure of a Sir2-like enzyme in complex with an acetylated peptide substrate has been solved, and provides the first glimpse into the mechanism of substrate recognition by this highly conserved family of enzymes.

Posttranslational modification of proteins by acetylation on lysine residues has been intensely studied over the past 10 years. Much of this work has been focused on acetylation of histones and other transcriptional regulators in eukaryotes—in fact, it would probably be fair to say that studies of histone acetylation have brought chromatin to the fore of the transcription field. Thanks to chromatin researchers, we now know a great amount about various enzymes that acetylate and deacetylate lysine residues [1, 2]. Most of these enzymes seem to have functions that are restricted to chromatin modification and transcriptional control in eukaryotes. Recently, however, much attention has been focused on a new class of deacetylase enzymes: the Sir2 family. These enzymes promise to give protein acetylation a much broader appeal.

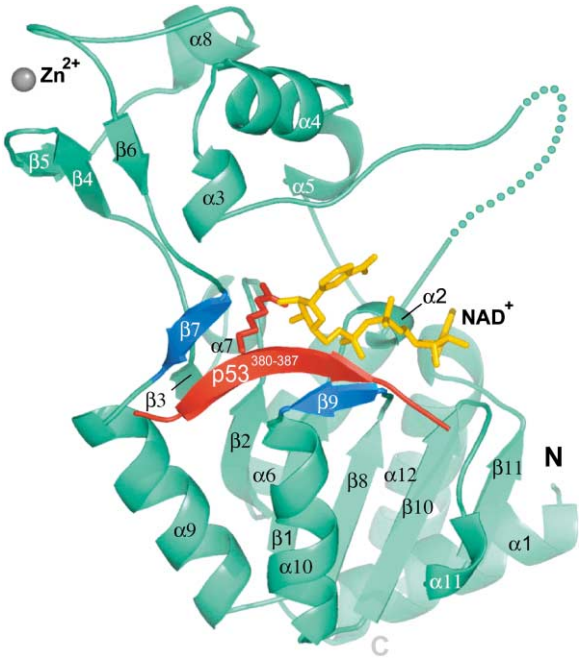
Proteins related to Sir2 have also been called sirtuins or class III histone deacetylases. However, it has become increasingly clear that many of these enzymes

have nothing to do with histones in vivo. The founding member of this family, the budding yeast Sir2 (*s*ilent *i*nformation *r*egulator 2), is in fact involved in chromatin-based gene silencing [3]. But, Sir2-like enzymes are conserved throughout evolution and thus are present in many organisms that do not even have histones (such as prokaryotes and some archaea, for example). This is in contrast to class I and II histone deacetylases (also called HDACs), which have only been found in eukaryotes. The biological functions of most Sir2 enzymes, and the identities of their substrates, are unknown. It is intriguing to note, however, that several distantly related Sir2 family members have been implicated in life span regulation and/or cell survival [4].

Another striking feature of Sir2-like enzymes is their enzymatic reaction mechanism. HDAC family enzymes catalyze a simple hydrolysis of acetyl-lysine residues to yield lysine and free acetate. By contrast, Sir2 deacetylation requires a cosubstrate, NAD [4, 5]. The NAD is actually consumed during the reaction, and, rather than liberating free acetate, these enzymes generate a novel metabolite, 2′-O-acetyl-ADP-ribose [6–8]. It is not clear why an energetically favorable reaction such as deacetylation would need to be coupled to breakage of a high-energy bond in NAD. It has been proposed that these enzymes act as metabolic "sensors," allowing different processes (such as life span extension) to be linked to the metabolic state of the cell [4]. Possible functions of the O-acetyl-ADP-ribose product are also unknown.

X-ray crystal structures of the archaeal Sir2-Af1 enzyme bound to NAD, as well as the unliganded human SIRT2, have recently been solved [9, 10]. The catalytic core of Sir2 enzymes consists of two domains: a large domain similar to the Rossmann fold, which is present in many NAD(H)/NADP(H) binding proteins, and a smaller domain that coordinates a zinc atom. In the Sir2-Af1-NAD structure, NAD lies in a groove between the two domains. The size of the groove could have accommodated an acetyl-lysine substrate as well, but the location of the active site could not be pinpointed. The absence of an acetylated substrate in the structure also left unresolved the issue of how different Sir2 family members might recognize different acetylated substrates.

In the September issue of *Molecular Cell*, Avalos et al. present the X-ray crystal structure of the archaeal Sir2-Af2 enzyme bound to an acetylated peptide substrate [11]. The peptide is derived from the regulatory domain of the p53 tumor suppressor protein, which is a good substrate for the enzyme in vitro but is, of course, never seen by the enzyme in cells. However, in this case, the use of a nonphysiological substrate helps to emphasize the key aspects of substrate binding revealed by the structure. The acetyl-lysine side chain on the peptide inserts into a tunnel on the enzyme that is in the cleft between the Rossmann fold and the zinc binding domain. The tunnel is lined with highly conserved hydrophobic residues Ile102 (in a loop C-terminal to β2), Phe165 (in a loop N-terminal to β7), and Val196 (in β9) (see Figure). The N-acetyl moiety is positioned adjacent to the NAD binding site observed in the previous structure, and is close to His118 (in a loop between β3 and β4), an invariant residue known to be required for catalysis. Other highly conserved or invariant residues,



Representation of the Structure of Sir2-Af2 in Complex with an Acetylated p53 Peptide

The structured portion of the p53 peptide substrate including the acetyl-lysine side chain is shown in red, and the β7 and β9 helices in Sir2-Af2, which form a β sheet with the p53 peptide (called the β staple), are shown in blue. The location of NAD, shown in yellow, is from the Sir2-Af1-NAD structure (open conformation) [9]. Courtesy of Jose Avalos and Cynthia Wolberger.

Val163, Gly166, Glu167, and Leu169 (all in or adjacent to β7), are involved in anchoring the substrate to the enzyme. Remarkably, all of the contacts between these residues and the substrate are hydrogen bonds between the peptide backbones of enzyme and substrate. In fact, the backbone of the substrate enters into an extended β sheet structure with the enzyme that the authors call a "β staple." This implies that any acetylated lysine that is within a stretch of amino acids flexible enough to form the β staple is a potential substrate for Sir2 enzymes. This view is consistent with the apparent lack of specificity displayed by some Sir2 family members in vitro, notably the yeast HST2 and the archaeal proteins Sir2-Af1 and Sir2-Af2. However, the authors also show that mutation of 3 nonconserved residues near the acetyl-lysine binding pocket can alter the substrate preference of Sir2-Af2, albeit only subtly (4- to 5-fold difference in reaction rate). Thus, different family members may have certain intrinsic substrate preferences that are of biological importance. The preference of the yeast Sir2 protein for lysine 16 of histone H4, a site that is important for gene silencing in vivo, may be one such example. At the same time, it seems very likely that the bulk of substrate preference for these enzymes in vivo will be guided by protein-protein interactions that do not involve the catalytic domain.

A comparison of the Sir2-Af2 peptide structure to previous Sir2 family structures shows several significant conformational differences. First, the position of the zinc

binding domain relative to the larger Rossmann fold is radically different in the three structures. This rotation of the zinc binding domain causes large changes in interatom distances in the different structures: up to a 10.8 Å difference between Sir2-Af2 peptide and Sir2-Af1-NAD, and up to a 14.3 Å difference between Sir2-Af2 peptide complex and SIRT2. Second, a loop that extends from the Rossmann fold domain and contacts NAD in the Sir2-Af1-NAD structure is disordered in the Sir2-Af2 peptide structure (indicated by a dotted line in the Figure). Since this loop is the most highly conserved stretch of amino acids in the Sir2 family, it is likely that the presence of NAD causes this loop to adopt a more ordered conformation. Finally, a different loop region that contains residues that contact the peptide substrate backbone and acetyl-lysine in Sir2-Af2 peptide (β7; called the FGE loop; see Figure) is shifted up to 8.7 Å to a more open conformation in the unliganded SIRT2 structure. The flexibility of the FGE loop is particularly interesting, since this loop seems to be central to the conformation of other parts of the molecule. In addition to making hydrogen bonds with the acetylated peptide, this loop has van der Waals contacts with helix α3, immediately adjacent to the NAD binding loop described above. There is also a salt bridge between Glu167 in the FGE loop and Lys159 in the zinc binding domain (in β6), and there is a connection to the putative active site via a hydrogen bond to His118. It is therefore tempting to speculate that the differences in conformation between the structures are all directly related to the presence of different bound substrates, and are mediated by the FGE loop. For example, one might imagine that binding of NAD to an unliganded enzyme would induce closure and/or ordering of the NAD binding loop, which would then cause the FGE loop to shift to the more closed conformation appropriate for peptide binding. Movement of the FGE loop could in turn explain the different positions of the zinc binding domain in the various structures.

Current models of the Sir2 reaction mechanism predict a nucleophilic attack on C1′ of the nicotinamide ribose by the carbonyl oxygen of the N-acetyl group of the substrate [8]. The proximity of the acetyl-lysine moiety to His118 in the Sir2-Af2 peptide structure could make the N-acetyl group a better nucleophile, in agreement with the proposed mechanism. An approximate location of the active site can be obtained by modeling the NAD structure from the Sir2-Af1-NAD crystal into the Sir2-Af2 peptide structure (see Figure). This picture of the active site is still somewhat imperfect because of steric clash between the N-acetyl group on the substrate and the 2′-hydroxyl group of NAD. Thus, it seems likely that the enzyme needs to sample even more conformations to form a productive enzyme-NAD-substrate ternary complex.

The idea that Sir2 enzymes undergo conformational movements during catalysis is appealing, since it might help explain how the energy expended by breakage of the C-N bond in NAD is used. What might such movement be doing? Conformational rearrangements may be crucial for some aspect of the deacetylation reaction mechanism, such as allowing tight binding to substrates or driving product release. It is also possible that the movement by the enzyme is doing work, allowing deacetylation to be coupled to another process that requires energy. Assembly of silencing complexes on chromatin could be one such process.

Though we now understand a great deal about the structural aspects of substrate binding by the Sir2 family of deacetylases, it remains to identify the in vivo targets of these enzymes. Given the evolutionary conservation of Sir2 family members, elucidation of their biological functions will undoubtedly uncover new roles for protein acetylation, be it on chromatin or elsewhere.

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The ABCDs of Periplasmic Copper Trafficking

The structure of the CopC protein of *Pseudomonas syringae* pathovar *tomato* provides fascinating clues, not only to its role in the periplasmic space in copper resistance, but also to features important for copper trafficking and homeostasis that may be conserved in a variety of biological systems.

Copper is a transition metal essential for the activity of a wide variety of enzymes such as cytochrome c oxidase, Cu,Zn superoxide dismutase, and multicopper ferroxi-