to expose a new hydrophobic patch that in the dimeric structure buries 890 Å^2 of surface area. The hydrophobic patch is conserved throughout this family of response regulators, which suggests that it may be important for biological function. Activation would therefore require significant flexibility in the hinge or linker region and loss of interdomain contacts to allow the symmetric head-to-head dimer to form between receiver domains while accommodating the asymmetric head-to-tail structure formed by the DNA binding domains.

This work extends a recent publication from the Stock laboratory on another activated OmpR/PhoB family member, ArcA, and may be generally applicable throughout this family (Toro-Roman et al., 2005). Taken together, these findings support the idea that upon phosphorylation these proteins form an active dimer along the α4-β5-α5 surface. However, important questions remain. Does the effector domain or DNA binding influence how these proteins dimerize in vivo? How does the interdomain linker region facilitate the nonsymmetrical packing of the domains in the activated structure? The answers to these and other questions will lead to a fuller understanding of the molecular mechanism of response regulator activation and will alleviate the fears of all phosphorylation phobes that this mechanism will never be unraveled.

William R. McCleary
Microbiology and Molecular Biology Department
Brigham Young University
Provo, Utah 84602

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Evolution of NAD Biosynthetic Enzymes

Two research groups have solved crystal structures of nicotinic acid phosphoribosyltransferase (PRTase) and made the argument that PRTases in three distinct pathways of nicotinamide adenine dinucleotide (NAD) biosynthesis evolved from a common ancestor (Shin et al., 2005; Chappie et al., 2005).

NAD has been central to many of the greatest discoveries in the biological sciences. In the latter half of the 19th century, Louis Pasteur popularized the study of fermentation of sugars by wine yeast (Pasteur, 1860). Pasteur's vitalist theories of fermentation were tenable only until the development of a high-tech instrument by Eduard Buchner: the sintered glass funnel. Once Buchner produced cell-free extracts that supported fermentation (Buchner, 1897), the mechanism of fermentation could be studied by biological chemists. This not only allowed a reductionist analysis of glycolysis, but was also a catalyst for evolution of the meaning of a word. Wilhelm Kuhne had coined the term "enzyme" for the substance responsible for glycolysis. However, those on both sides of the debate about vitalism used the term. For Pasteur, enzyme simply meant "in yeast," because he believed that glycolysis was a series of reactions in yeast cells. For those who believed and ultimately demonstrated that glycolysis is a function of subcellular components, enzyme meant something in yeast that could also be taken "out of yeast" or "from yeast." To emphasize the distinct physical nature of enzymes, Buchner, Harden, and their contemporaries began using the term "zymase," meaning an enzyme fraction from yeast or another cellular source (Barnett, 2003).

Arthur Harden's Nobel Prize-winning contribution 100 years ago was to separate a zymase fraction from a cozymase fraction by using a dialysis membrane and to show that reconstitution of cell-free glycolysis required the high molecular weight, heat-labile zymase plus the low molecular weight, heat-stable cozymase (Harden and Young, 1905), which we now understand to contain NAD and ATP-magnesium. Years later, Hans von Euler and Otto Warburg independently determined the chemical structure of NAD (Schlenk and von Euler, 1936; Warburg and Christian, 1936), and Conrad Elvehjem identified nicotinic acid and nicotinamide as two vitamin precursors of NAD (Elvehjem et al., 1938). Subsequently, the enzymologists Jack Preiss and Philip Handler identified the three enzymes and two stable metabolic intermediates required to synthesize NAD from nicotinic acid (Preiss and Handler, 1958). However, it was not until peptide and nucleotide sequences could be assigned to NAD metabolic enzymes that anyone could profitably think about the evolution of NAD metabolism.

Different cells utilize different strategies to make NAD. Many, but not all, bacteria and many, but not all, eukaryotes possess aerobic de novo pathways to make NAD from either aspartic acid or tryptophan. De novo NAD biosynthesis results in a common intermediate with the Preiss-Handler pathway: nicotinic acid mononucleotide (NaMN). In fact, formation of NaMN from the de novo pathway and formation of NaMN from the Preiss-Handler pathway both utilize a PRTase, an enzyme that transfers a phosphoribosyl group to a base from 5-phosphoribosyl-1-pyrophosphate. In de novo pathways to NAD, the substrate is quinolinic acid, and in the Preiss-Handler pathway, the substrate is nicotinic acid. Thus, a cell that has oxygen and expresses a de novo pathway uses quinolinic acid PRTase to make NaMN, whereas a cell that imports or salvages nicotinic acid uses nicotinic acid PRTase to make NaMN. Once NaMN is produced, two more enzymes are required to complete the synthesis of NAD: NaMN adenylyltransferase and NAD synthetase (Preiss and Handler, 1958).

Though NAD has been understood since von Euler's time to be a coenzyme for hydride transfer enzymes that interconvert NAD and NADH, NAD is also a substrate for glycohydrolases that consume NAD, producing nicotinamide and an ADP ribosyl product. NAD glycohydrolases are fascinating enzymes such as Sirtuins (Imai et al., 2000), which deacetylate protein lysine modifications with consumption of NAD, and cyclic ADPribose synthases and poly(ADPribose)polymerases (Ziegler, 2000), both of which consume an equivalent of NAD for every ADPribosyl moiety cyclized or polymerized.

Different cells have different ways to salvage nicotinamide. If a cell expresses a nicotinamidase gene, then nicotinamide produced by NAD glycohyrolases is converted to nicotinic acid. The cell would then need the three enzymes of the Preiss-Handler pathway to convert nicotinic acid to NAD. Alternatively, some cells encode a nicotinamide PRTase that converts nicotinamide to NMN for subsequent adenylylation to NAD.

How did the enzymes involved in NAD biosynthesis come about? Trivially, all known quinolinic acid PRTases are related to each other. More significantly, the three PRTases in NAD biosynthetic pathways are all related (Shin et al., 2005; Chappie et al., 2005). On the basis of structural data, Stevens and coworkers specifically propose that quinolinic acid PRTase gave rise to nicotinic acid PRTase, which, in turn, evolved into nicotinamide PRTase by rearrangements, mutations, and selection (Chappie et al., 2005). Although structural and kinetic data alone might have been compatible with convergent evolution, the primary sequence data provide a compelling case that these enzymes arose divergently from a common ancestor (Shin et al., 2005; Chappie et al., 2005).

Apart from the sequence and structural data used to argue that quinolinic acid PRTase was the predecessor of the two other NAD biosynthetic enzymes (Chappie et al., 2005), what we know about metabolism makes this a strong expectation. Because we know of no abiotic source or de novo pathway for nicotinic acid or nicotinamide (Elvehjem et al., 1938), or nicotinamide riboside (Bieganowski and Brenner, 2004), we would expect that ancestral cells made NAD de novo and we would expect a selection for salvage enzymes occurred only after specific or nonspecific NAD degradative pathways generated salvageable products. But the logic of a quinolinic acid PRTase gene as the ancestor of nicotinic acid and nicotinamide PRTase genes becomes troubling when one considers that de novo pathways in most familiar organisms contain oxygen-dependent steps. If early life on earth was anaerobic, how was de novo NAD biosynthesis accomplished? Remarkably, genes encoding homologs of the aerobic pathway from aspartic acid have been found in the anaerobic hyperthermophilic archaeon *Pyrococcus horikoshii* (Sakuraba et al., 2002) and in many other anaerobes. This suggests that an anaerobic de novo pathway could plausibly be ancestral and that the enzymology of de novo NAD biosynthesis may have adapted to and become dependent on oxygen in aerobic evolution.

For those of us who practice biology and seek to understand how life as we know it came to be, these crystal structures and the amino acid sequence and metabolic data that underlie them provide insights into how living things evolved over four billion years.

Charles Brenner
Department of Genetics
Dartmouth Medical School,
Lebanon, New Hampshire 03756

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