Reversible phosphorylation: a birthday tribute to Herb Tabor

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Herb Tabor was the Editor-in-Chief of the Journal of Biological Chemistry (JBC) spanning the years 1971–2010. This year, Herb turns 100. What do you give a person turning 100? Our answer to this question was to dedicate two of our favorite JBC papers to Herb. Both of these papers focus on reversible phosphorylation, which we briefly review. In addition, we delve into a new finding that centers around a novel family of secreted kinases, suggesting that there are many new and exciting discoveries yet to explore.

A living legend turns 100! Herb Tabor published his first paper in the JBC in 1943, and he has been the face of the JBC throughout our entire scientific careers. Jack Dixon served on the Editorial Board of the JBC and saw first-hand Herb’s style and class at running the Journal. Herb was always prepared for anything that came up during those editorial board meetings. Jack also had a chance to interact with Herb the year he served as the President of the American Society of Biochemistry and Molecular Biology (ASBMB) in 1996. This was a critical time for the JBC, as it was the first journal in the field to transition from a “paper-only” format to an electronic format. Herb was masterful in shaping this transition, and his careful and insightful eye made it possible for the JBC to make this pioneering move to a highly accessible electronic format ahead of all the other journals in the field.

Over the course of our careers, the laboratory has focused on the field of reversible phosphorylation. In this birthday wish, we want to briefly highlight the history of reversible phosphorylation and underscore seminal papers published in the JBC. Along the way, we will discuss two favorite papers from Jack’s laboratory that were published in the JBC. Both papers focus on our work with protein phosphatases: specifically, our discovery of the catalytic mechanism of the protein-tyrosine phosphatases (1), and our work on the tumor suppressor, phosphatase and tensin homolog (PTEN) (2). We want to dedicate these papers to Herb to commemorate his 100th birthday. Finally, we will highlight our laboratory’s recent discovery of a novel family of kinases that keeps us excited about the surprises in store for this fascinating field (3).

Aspects of reversible phosphorylation: a brief history

The first proteins described to contain covalently bound phosphate, casein and phosvitin, were identified by Lipmann and Levene in the early 1930s (4, 5). It is noteworthy that the first occurrence of “serine phosphoric acid,” i.e. phosphoserine, was published in the JBC. Both casein and phosvitin contain many phosphoserine residues and are important nutritionally in either early embryonic development or in feeding of infant mammals.

Fast-forward 10 years, the first inroads to the concept that phosphorylation was a dynamic reversible process came from the work of Carl and Gerti Cori and Arda Alden Green on glycogen metabolism. In two JBC papers published in 1943 and 1945, they set the groundwork for the emerging field of regulation by reversible phosphorylation by demonstrating that phosphorylase, which catalyzes the first step in glycogen breakdown, existed in inactive and active forms that they named phosphorylase a and phosphorylase b (6, 7). At the time, the nature of the conversion of phosphorylase a to b, the first “interconvertible enzyme” system was not known. Indeed, it would be another 10 years before the laboratories of Sutherland and co-workers (8) and Fischer and Krebs (9) would unravel this mystery. Sutherland’s laboratory studying liver phosphorylase and Fisher and Krebs working on the muscle equivalent were in a race to publish the first article of how reversible phosphorylation regulates enzymatic activity. During the years 1955–1958, these scientists discovered that the active form of phosphorylase is a phosphoenzyme and that the conversion to the inactive form involves enzyme-mediated dephosphorylation (Fig. 1). Several of these ground-breaking experiments were published in the JBC (8, 9). Later, it was evident that phosphorylation increased the activity of enzymes involved in glycogen breakdown while decreasing the activity of enzymes responsible for glycogen synthesis. This was accomplished by kinase cascades, one of the first of which was published by Walsh et al. (10) in JBC where they described the activity of an adenosine 3′,5′-monophosphate-dependent protein kinase that could activate phosphorylase kinase and suggested that it be called phosphorylase kinase kinase. This reciprocal relationship between the enzymes involved in glycogen synthesis and breakdown is now known to not only hold for enzymes involved in biosynthetic pathways, but also enzymes and proteins involved in processes as diverse as contractility, secretion, membrane transport, transcription, and many more. Indeed, reversible phosphorylation is recognized as one of the established principles for regulating biological processes.
serine, the enzymatic activity of the phosphatase was completely eliminated. Mutations of other residues in the phosphatase reduced the activity somewhat but not entirely. This result plus additional chemical studies indicated that the protein-tyrosine phosphatase forms a covalent thiol phosphate linkage between the catalytic cysteine residue and the phosphate (Fig. 2). This mechanism of catalysis was distinct from that of the serine/threonine phosphatases. The active site of the PTPases is composed of the highly conserved residues, HC(X)5R, surrounding an essential Cys residue that served as the key catalytic residue (Fig. 2). The fact that other PTPases from pathogenic bacteria to humans have the conserved sequence at their active sites suggests that this family of enzymes uses a common catalytic mechanism.

Substrate for the tumor suppressor PTEN/MMAC1

As mentioned above, kinases were getting attention as promoters of cancer, so we and others questioned whether the phosphatases could possibly be tumor suppressors. Each new phosphatase gene that was cloned and expressed was examined as a possible tumor suppressor, but only negative results were obtained until two groups (14, 15) published that a new phosphatase, known as PTEN or MMAC1, had all the key features to suggest that it was a tumor suppressor. However, the PTEN phosphatase had very low enzyme activity toward phosphorylated protein substrates. Surprisingly, it displayed good activity toward the highly negatively charged, multiply phosphorylated substrate such as $p$-nitrophenyl phosphate, the phosphatase itself became transiently labeled with $^{32}$P. When an essential cysteine residue of the protein-tyrosine phosphatase was mutated to serine, the enzymatic activity of the phosphatase was completely eliminated. Mutations of other residues in the phosphatase reduced the activity somewhat but not entirely. This result plus additional chemical studies indicated that the protein-tyrosine phosphatase forms a covalent thiol phosphate linkage between the catalytic cysteine residue and the phosphate (Fig. 2). This mechanism of catalysis was distinct from that of the serine/threonine phosphatases. The active site of the PTPases is composed of the highly conserved residues, HC(X)5R, surrounding an essential Cys residue that served as the key catalytic residue (Fig. 2). The fact that other PTPases from pathogenic bacteria to humans have the conserved sequence at their active sites suggests that this family of enzymes uses a common catalytic mechanism.

Catalytic mechanism of protein-tyrosine phosphatases

The Dixon laboratory became involved in reversible phosphorylation in the late 1980s. By this time, the field had progressed to where there was a great deal of excitement about tyrosine kinases and the roles they play in cancer. So, it was natural to ask about the phosphatases that could dephosphorylate phosphotyrosine. Prior to that, work had focused on the serine and threonine phosphatases involved in glycogen metabolism and other processes mentioned above (11). A breakthrough in the study of tyrosine phosphatases occurred when Harry Charbonneau, Nick Tonks, and Ken Walsh, postdoctoral fellows in Ed Fischer’s laboratory, published a paper describing the amino acid sequence of a portion of PTP1B, the first tyrosine phosphatase to be purified and shown to be specific for the amino acid sequence of a portion of PTP1B, the first tyrosine phosphatase (PTPase) of 432 amino acids showing 97% sequence identity to the homologous residues of PTP1B (13). We named this phosphatase PTP1 because it was the first tyrosine phosphatase isolated from brain. My (Jack’s) postdoctoral fellow, Kun-Liang Guan, and I co-authored one of my favorite JBC papers on PTP1 (1). In this paper, we demonstrated the mechanism that the protein-tyrosine phosphatases use in catalysis. Basically, we developed a system to produce a substantial amount of a recombinant protein-tyrosine phosphatase in Escherichia coli using a glutathione S-transferase tag. When the purified protein-tyrosine phosphatase interacted with a $^{32}$P-labeled substrate such as $p$-nitrophenyl phosphate, the phosphatase itself became transiently labeled with $^{32}$P. When an essential cysteine residue of the protein-tyrosine phosphatase was mutated to serine, the enzymatic activity of the phosphatase was completely eliminated. Mutations of other residues in the phosphatase reduced the activity somewhat but not entirely. This result plus additional chemical studies indicated that the protein-tyrosine phosphatase forms a covalent thiol phosphate linkage between the catalytic cysteine residue and the phosphate (Fig. 2). This mechanism of catalysis was distinct from that of the serine/threonine phosphatases. The active site of the PTPases is composed of the highly conserved residues, HC(X)5R, surrounding an essential Cys residue that served as the key catalytic residue (Fig. 2). The fact that other PTPases from pathogenic bacteria to humans have the conserved sequence at their active sites suggests that this family of enzymes uses a common catalytic mechanism.

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in the absence of insulin stimulation. We went on to show that recombinant PTEN catalyzed dephosphorylation of PtdIns(3,4,5)P$_3$ specifically at position 3 on the inositol ring and exhibited 3-phosphatase activity toward a second substrate, inositol 1,3,4,5-tetrakisphosphate. Our results raised the possibility that PTEN acts in vivo as a phosphoinositide 3-phosphatase by regulating PtdIns(3,4,5)P$_3$ levels. As expected, the C1245 mutant of PTEN was incapable of catalyzing dephosphorylation of PtdIns(3,4,5)P$_3$, consistent with the mechanism observed in protein-tyrosine phosphatase–catalyzed reactions. This paper, published as a Communication in the JBC, has been cited more than 3000 times (2).

### The future of reversible phosphorylation

There are thousands of papers published on kinases and reversible phosphorylation, which might suggest that all the important findings must surely have been made. I would argue differently, and I will provide several recent unexpected findings from the laboratory. Specifically, I return to the beginning of both this article and the reversible phosphorylation field to pose the following question: “What is the identity of the kinase(s) that phosphorylates the secreted proteins casein and phosvitin?” In 2002, Manning, Hunter, and colleagues (18) catalogued the protein kinase complement of the human genome identifying ~518 putative kinase genes. We were puzzled by the fact that most of these kinases were localized to the cytoplasm or nucleus, although none were known to reside in the secretory pathway. In direct opposition to this, proteomics suggested to a new postdoctoral fellow, Vincent Tagliabracci, that he look for mammalian proteins that were related to fjx by amino acid sequence alignment with the hope of identifying new kinases in the secretory pathway. As a working hypothesis, we set out to look for fjx-like proteins that had NH$_2$-terminal signal sequences that could target the proteins to the lumen of the endoplasmic reticulum. Vinnie uncovered a small family of proteins that had been assigned the names Fam198A, Fam198B, Fam20A, Fam20B, Fam20C, Fjx, Fam198A, and Fam198B are shown.

![Figure 3. PTEN catalytic reaction](image)

PTEN catalyzes the removal of a phosphate from the 3’-position of PtdIns(3,4,5)P$_3$.

![Figure 4. Novel family of secreted kinases](image)

A family tree of secreted kinase homologues that have now been identified. A schematic diagram of a typical secreted kinase. These kinases have NH$_2$-terminal signal peptides as well as kinase domains located at their COOH termini. Fjx, Fam20A, Fam20B, Fam20C, Fjx, Fam198A, and Fam198B are shown.
Following this revelation, Junyu Xiao, another postdoctoral fellow in the laboratory, produced enough of the recombinant Fam20C protein that he was able to crystallize it and determine its structure at atomic-level resolution (26). Surprisingly, it harbored many of the same structural elements found in protein kinase A (PKA), the first kinase structure published by Susan Taylor and colleagues in the early 1990s (27), with the addition of several unique regions. These similarities demonstrated that despite pronounced sequence divergence, the core folds are maintained in these novel kinases and throughout the kinase family.

The other surprise was that mutations in Fam20C cause Raine syndrome, a deadly disease resulting in bone dysplasia characterized by osteosclerosis and ectopic calcifications (28, 29). Most of the mutations in Raine syndrome patients do not produce an active kinase and are often lethal neonatally, whereas the nonlethal mutations produce a kinase with low levels of activity. Therefore, we surmised that Fam20C was important in the development of bones and other calcified structures such as teeth. But, when we analyzed the secreted phosphoproteome of a variety of cell types, including osteosarcoma (U2OS) and liver (HepG2) and breast cancer cells, we were surprised that Fam20C generated the majority of the extracellular phosphoproteome, with hundreds of proteins phosphorylated on the Ser residue of the SXE motif (30). It comes as no surprise that the Fam20C kinase has many substrates and that mutations in the substrate that alter SXE residues in the phosphorylation motif could lead to disease. This is indeed the case. We have uncovered several examples of this that we are studying in greater detail (31). As always, it is exciting to anticipate the next unexpected path of discovery.

I (Jack) want to close by saying what a pleasure it has been to interact with Herb for over 40 years, my entire scientific career. A hundred years is something special, and you, Dr. Tabor are also something special. Happy Birthday and best wishes.

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