Catching protein polyphosphorylation in the act

DOI 10.1074/jbc.H120.012632

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Lysine polyphosphorylation (K-PPn) is a relatively new post-translational modification, the full targets and functional consequences of which are unknown. A critical problem in the study of endogenous K-PPn of proteins in the yeast model system is that its nonenzymatic nature and its susceptibility to polyphosphatases make it potentially susceptible to artifacts during extraction. A new study confirms that K-PPn modifications can be altered during sample handling, provides new insights into the mechanism of K-PPn, and develops a yeast model strain, devoid of both vacuolar polyP and polyphosphatases, that allows detection of authentic endogenous K-PPn.

PolyP is a polymer of three to hundreds of orthophosphates, connected by high-energy phosphoanhydride bonds and present in most prokaryotic and eukaryotic cells (1). PolyP is predominantly accumulated in acidic organelles, like the yeast vacuoles and acidocalcisomes (2), but it is also present in other organelles and even inserts into the plasma membrane of eukaryotic cells (3). The enzymes involved in polyP metabolism, including Vtc4 (vacuolar transporter chaperone 4) and a suite of polyphosphatases, are known in the budding yeast Saccharomyces cerevisiae, which has made this organism the best model system for further study. The enzymes involved in its synthesis are also known in bacteria and archaea (polyP kinases, Ppkks), algae, trypanosomes, and apicomplexan parasites (Vtc4) (1, 3), whereas the enzymes that degrade polyP, the endopolyphosphatases (Ppn) and exopolyphosphatases (Ppx), have also been identified in mammals (1, 3). Multiple functions have been attributed to this polymer, from being an inorganic chaperone in bacteria (4) to modulation of blood coagulation in mammals (5). However, it is not clear that we know the full scope of polyP activities or how this seemingly simple polymer accomplishes such a diversity of tasks. An improved understanding of these roles would not only increase our appreciation of polyP but might shed new light on the function of other cellular biopolymers.

In previous work, Azevedo et al. (6) identified yet another function for polyP in their discovery that it can nonenzymatically attach to lysines of PASK (polycacidic serine and lysine (K)) domains in yeast, creating a new post-translational modification (PTM). The identification of polyphosphorylated proteins relied on the decrease in their mobility (electrophoretic shift) on NuPage gels and was initially demonstrated in two nuclear proteins, DNA topoisomerase 1 (Top1) and its interacting partner, nuclear signal recognition 1 (Nsr1) (6). Lysine polyphosphorylation (K-PPn) of these proteins resulted in down-regulation of their interaction and reduced enzymatic activity of Top1 (6). This K-PPn was later described in other nuclear proteins involved in ribosomal biogenesis (7) as well as in proteins with other localizations and functions (7–9), including human proteins (7, 8).

The nonenzymatic nature of K-PPn makes it difficult to be confident that proteins extracted from lysed cells are endogenously polyphosphorylated, rather than artifically polyphosphorylated as a result of their contact with polyP released from organelles. This is more relevant when studying K-PPn in yeasts, which possess large amounts of polyP in their vacuoles (6). The yeast vacuole is also rich in endopolyphosphatases, Ppn1 and Ppn2, which could hydrolyze protein-bound polyP (6) and then artifically reduce K-PPn. To investigate whether it is possible to discriminate between endogenous from artificial changes in K-PPn in yeast, Azevedo et al. (10) now test these possibilities, leading them to create a strain appropriate to study this process in a more physiological context and provide additional information on this PTM.

In their experiments, Azevedo et al. (10) first confirmed that K-PPn of Top1 and Nsr1 could be increased after cell lysis, as they observed an increased electrophoretic shift on the NuPage gels when using WT cells, mutants that generate more polyP (inositol polyphosphate kinase mutant, vipΔ, and the ppn1Δppn2Δppx1Δ mutant), or by the addition of exogenous polyP of different lengths (P45 and P100), but not when using mutants deficient in polyP synthesis (vtc4Δ). The authors demonstrated this increase is due to the modification of new Lys residues rather than to alteration of previously modified residues (Fig. 1). Mutation of all acidic residues (Asp and Glu) of the PASK domain of Top1 abolished this shift, indicating that these residues are necessary for this PTM to occur, possibly by stabilizing a network of divalent cations to coordinate the polyP chain. Azevedo et al. (10) then analyzed how the release of endopolyphosphatases upon cell lysis could affect K-PPn. When lysates from Ppn1- and Ppn2-deficient cells were prepared, a higher mobility of Top1 and Nsr1 in NuPage gels was...
observed, indicating that the proteins were highly polyphosphorylated. These changes were more evident with Nsr1. The impact of a deficiency in the other polyphosphatases known to be present in yeast, Ppn1 and diadenosine triphosphoinositol polyP phosphohydrolase 1 (Ddp1), was not so evident, suggesting that the vacuolar endopolyphosphatases, Ppn1 and Ppn2, have a more important role in depolyphosphorylation of proteins in yeast lysates (Fig. 1). However, some remaining depolyphosphorylation did occur even when all four polyphosphatases were knocked out, indicating that other cellular phosphatases are also involved. Finally, Azevedo et al. (10) targeted Ppx1 to the vacuole of yeast knockouts for Ppn1 and Ppn2 to create a mutant cell line with no vacuolar polyP, as it is degraded by Ppx1, and no relevant polyphosphatases, as only the endopolyphosphatases Ppn1 and Ppn2 were found to significantly break the protein–phosphate bond of Top1 and Nsr1. Interestingly, this strain retained a pool of nuclear polyP, allowing the unaltered endogenous polyphosphorylation of nuclear Top1, whereas Nsr1, which shuttles between the cytoplasm and the nucleus, was more sensitive to the loss of vacuolar polyP.

Some questions will need to be addressed in future studies. How is the nuclear polyP pool generated? One possibility, discussed by Azevedo et al. (10), is the presence of an additional endoplasmic reticulum localization of Vtc4. How general is yeast as a model system? For example, will the concentration of polyP and polyphosphatases released after disruption of mammalian cells similarly be sufficient to artifactual polyphosphate/depolyphosphorylate proteins? This could also be the case for several protists that also have large amounts of polyP in acidocalcisomes as well as conserved polyphosphatases (2). Could the model yeast strain developed be used to detect polyP phosphorylation of proteins from other species? If divalent cations are needed to coordinate the polyP chain and allow polyphosphorylation, would cell lysis in the presence of chelators, such as EDTA, prevent artifactual polyphosphorylation? Perhaps chelators could also prevent Ppn1 and Ppn2 activity, as these enzymes require metals for catalysis. Some of these questions could be answered using the new model developed in this work.

In summary, the intelligent use of a variety of yeast mutants demonstrated that vacuolar polyP and endopolyphosphatases Ppn1 and Ppn2 can significantly affect the K-PPn of proteins upon cell lysis. The new model strain developed in this work should be useful to identify authentic endogenously polyphosphorylated proteins in yeast and contribute to our understanding of this novel posttranslational modification.

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