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Interfacing protein lysine acetylation and protein phosphorylation

Ancient modifications meet on ancient proteins

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Recognition that different protein covalent modifications can operate in concert to regulate a single protein has forced us to re-think the relationship between amino acid side chain modifications and protein function. Results presented by Tran et al.[13] demonstrate the association of a protein phosphatase (PP2A) with a histone/lysine deacetylase (HDA14) on plant microtubules along with a histone/lysine acetyltransferase (ELP3). This finding reveals a regulatory interface between two prevalent covalent protein modifications, protein phosphorylation and acetylation, emphasizing the integrated complexity of post-translational protein regulation found in nature.

Mass spectrometry based detection and quantitation of protein covalent modifications has dramatically altered our view of the prevalence and variety of amino acid side chain alternations that exist on any given protein. Protein phosphorylation is now thought to be common to at least 70% of all proteins with most having multiple phosphorylation sites.[1] Several recent studies have cataloged protein lysine acetylation sites, revealing this modification as a common regulatory mechanism for diverse cellular processes.[2-6] Like phosphorylation, lysine acetylation is much more prevalent than originally thought, with similar trends likely to emerge for other modifications over time.[6] It is becoming clear that different modifications often do not work in isolation, as a combination of modifications often control the function of a protein. The idea that one protein may undergo multiple modifications concurrently and that these operate together originated from studies on histones, ultimately leading to the histone code hypothesis.[7] Many covalent modifications reside within sequence specific binding sites for domains or modules within other proteins and often recruitment of one protein controls other site modifications and protein recruitments.[8] Given the high level of different modifications of many proteins, the concept of a protein code is now emerging.[7]

The recent burst of information regarding widespread protein acetylation has lead to several keys studies demonstrating the intimate relationship between phosphorylation and acetylation. Glycogen phosphorylase was the first protein to be discovered as reversibly phosphorylated. In liver, it is dephosphorylated by glycogen targeted protein phosphatase one ($PP1G_L$). Human liver glycogen phosphorylase is acetylated at K470 and this enhances the recruitment of $PP1G_L$, which dephosphorylates and inactivates the enzyme.[9] Similarly, the adenosine monophosphate-activated protein kinase (AMPK) has been shown to be acetylated and the deacetylation of AMPK promotes interaction with its activating upstream kinase, LKB1.[10] The interplay of acetylation and phosphorylation has also been extended to prokaryotes where a systems biology approach confirmed widespread bacterial protein phosphorylation and acetylation, often on the same protein. Knockout of individual protein kinases or phosphatases resulted in altered lysine acetylation patterns.[11] A clear functional role for interacting modifications has also been demonstrated for human bromodomains that dock lysine acetylation motifs in target proteins. Comprehensive screens of bromodomain family members demonstrated that nearby phosphorylation or acetylation events near the bromo binding motif affects docking to these proteins.[12]

It is perhaps not surprising that mass spectrometry studies have found abundant protein covalent modifications in plants.[4,5] The recent work of Tran et al. has made a link between the key protein phosphatase PP2A and protein acetylation.[13] Starting with a biochemical affinity capture of microcystin sensitive protein phosphatases, this work cataloged a large number of phosphatase catalytic subunits[14] and known regulatory proteins.[15] Also uncovered were a number of novel phosphatase complex proteins including the lysine acetyltransferase ELP3 and deacetylase HDA14, known more generally as a histone acetyltransferase and histone deacetylase, respectively. These findings were able to demonstrate, based on sequence alignments, that *Arabidopsis thaliana* HDA14 is most like human HDAC6 and like its human counterpart can deacetylate α-tubulin at K40. Given the association of both ELP3 and HDA14 with microtubules we proposed that they function as the endogenous α-tubulin acetylating and deacetylating enzymes. Curiously, both co-purify on the phosphatase affinity matrix suggesting complex formation with a

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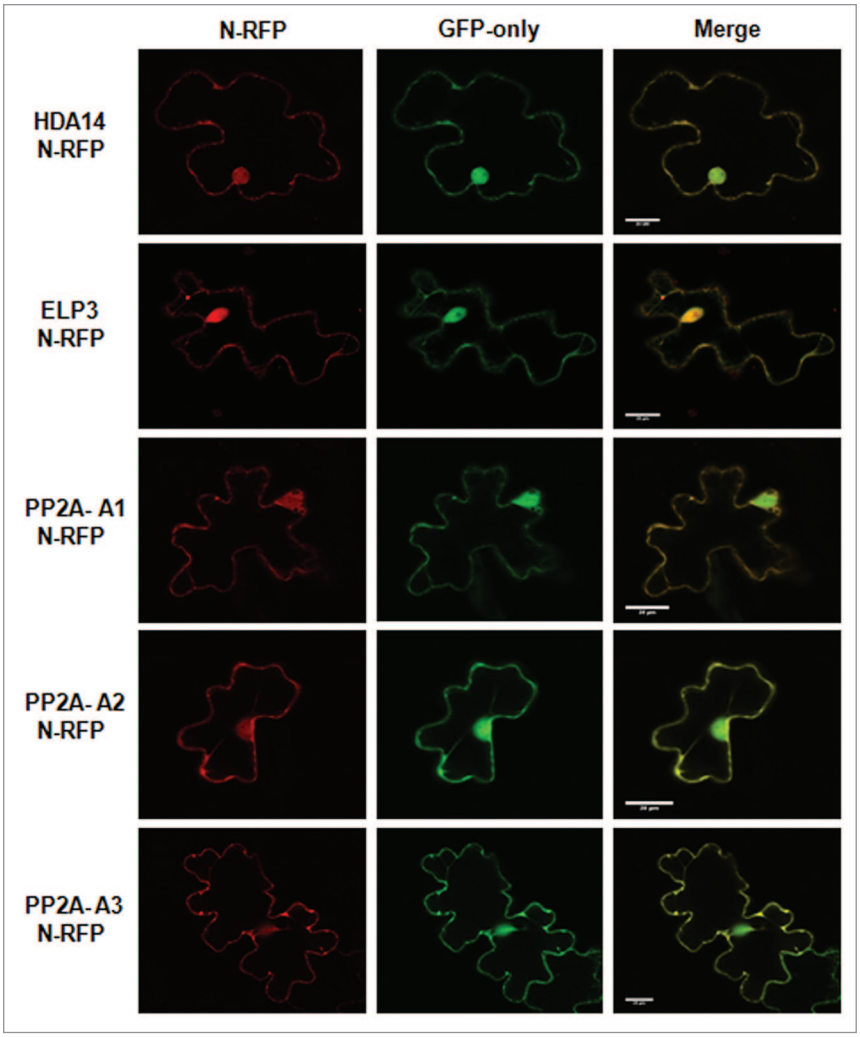

Figure 1. Cellular localization of N-terminal tagged HDA14, ELP3, PP2A-A1, A2 and A3 RFP constructs and fluorescent GFP-only marker construct p2FGW7 in *Vicia faba* leaves. All images are single slices of pavement cells obtained using a confocal laser scanning microscope (Leica). RFP images are on the left (red), GFP-only images in the center (green) and the merged image on the right (yellow). Constructs were generated and experiments performed as in Tran et al.[13] Scale bar = 20 μm.

phosphatase. As well, data revealed that HDA14 can interact directly with the scaffolding A2 subunit of plant PP2A, and along with ELP3, co-purify on taxol-stabilized microtubules. Currently it is unclear why the deacetylase associates with PP2A, but we speculate that it controls either the acetylation status of PP2A in addition to α-tubulin, or the phosphorylation status of HDA14 and/or ELP3.

The work of Tran et al.[13] also highlights another interesting point in the context of the now recognized high degree of protein phosphorylation and acetylation in plants. Plants, like humans, have relatively few phosphatase catalytic subunits, acetyltransferases and deacetylases. Contrary to protein kinases, the paradigm which has emerged for protein phosphatases is that they are relatively non-specific and maintain substrate specificity through their interactions with a vast array of regulatory proteins.[14,15] How then can a limited number of deacetylases and acetyltransferases acquire specificity? Likely it will come from a large number of additional binding partners. This is hinted at when we examine the distribution of HDA14, ELP3 and the PP2A scaffolding subunits. Although there are differences in distribution, all reside in both the nucleus and cytoplasm (**Fig. 1**) and likely have a vast range of substrates in each compartment. Future studies will undoubtedly uncover many regulatory partners for each. The work of Tran et al.[13] finally opens the door on the relationship between protein acetylation and phosphorylation in plants.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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