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HDAC6-Pack: Cortactin Acetylation Joins the Brew

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The reversible acetylation of lysine residues is an important posttranslational modification for the regulation of histones, transcription factors, chaperones, and microtubules. In a recent article in *Molecular Cell*, Zhang et al. (2007) describe a new target of reversible acetylation, the actin binding protein cortactin.

Cortactin is an 80–85 kDa protein, initially identified as a Src substrate, that localizes to regions of cells undergoing active membrane remodeling such as lamellipodia, growth cones, podosomes, invadipodia, and endocytic vesicles (Cosen-Binker and Kapus, 2006; Wu et al., 1991). Cortactin is composed of three major interaction domains: (1) an N-terminal acidic region (NTA) containing a DDW motif that binds the actin nucleating Arp2/3 complex; (2) a region following the NTA composed of 6.5 tandem repeats of a unique 37 amino acid sequence that binds F-actin; and (3) a Src homology 3 (SH3) domain at the C terminus that binds a number of proteins, including N-WASP, WIP, dynamin-2, MIM, CD2AP, Zo-1, SHANK2, FDG1, MLCK, and CBP90 (Cosen-Binker and Kapus 2006). Between the repeats and the SH3 domain is an α-helical domain of unknown function, a proline-serine-threonine-rich region that is the site of phosphorylation by PAK and ERK kinases and Tyr residues that are phosphorylated by Src family kinases. The long list of interacting proteins reflects the importance of cortactin in integrating signaling with the remodeling of the actin cytoskeleton at the cell membrane. Indeed, cortactin has recently been implicated in cancer metastasis on account of its increased expression in a variety of carcinomas.

Zhang et al. (2007) demonstrate that cortactin is acetylated in vivo and is a genuine substrate of histone deacetylase 6 (HDAC6), which directly interacts with the repeat region of cortactin via its two catalytic domains. Overexpression of HDAC6 in cells results in hypoacetylated cortactin, while inhibition of HDAC6 using siRNA- or HDAC6-specific inhibitors results in hyperacetylated cortactin. A second cytoplasmic deacetylase, SIRT2, which associates with HDAC6, may also be involved in cortactin deacetylation, as specific inhibition of this deacetylase also increased cortactin acetylation in vivo. The authors identify the p300-CBP-associated factor (PCAF) as a potential cortactin acetyltransferase. PCAF specifically acetylated the repeat region of cortactin, which was also found to behave as a HDAC6 substrate. There may be additional cortactin acetyltransferases, as knock down of PCAF did not reduce acetylated cortactin levels in cells.

Mass spectrometry showed that multiple lysines in the F-actin-binding repeat region of cortactin were acetylated. A mutant construct of cortactin in which each of the nine acetylated lysines were substituted with glutamine could still bind HDAC6 but was unable to be acetylated, suggesting that the repeat region is likely to be the major, if not only, region acetylated in cortactin. Using secondary structure prediction and computer modeling, the authors present a model where the acetylated lysine residues occupy loops connecting α helices within the repeat region, and they predict the existence of two "charged patches" that may be involved in binding F-actin. In fact, nonacetylated cortactin bound F-actin while acetylated cortactin did not. Progressively mutating more of the lysines to glutamine (making them uncharged to mimic acetyl-lysine) revealed a graded effect on the binding of cortactin to F-actin: mutation of fewer than three lysines had no effect, while mutation of four or more increasingly attenuated F-actin binding. A charge-preserving cortactin mutant in which all of the acetylated lysines in the repeat region were mutated to arginine bound F-actin, further demonstrating the importance of the charge state of the repeat region in F-actin binding.

Does acetylation of cortactin affect its function in vivo? Zhang et al. (2007) first demonstrate that growth factor stimulation or Rac activation, both of which are known to induce cortactin redistribution to the cell periphery, resulted in hypoacetylation of cortactin and translocation of cortactin to the cell periphery. Preventing deacetylation of cortactin blocked this redistribution. Interestingly, HDAC6 also relocated to the cell periphery under these conditions, suggesting that HDAC6 may also be regulated by Rac.

Using migration assays, the authors show that inhibition of HDAC6 decreased cell motility as expected from earlier studies (Haggarty et al., 2003; Hubbert et al., 2002; Matsuyama et al., 2002; Zhang et al., 2003). Critically, decreased motility depended on the hyperacetylation of cortactin as shown by stable cell lines expressing repeat region mutants that could not be acetylated. Conversely, cortactin acetylation-mimicking mutants did not rescue the migration defects of cells knocked down for cortactin. Zhang et al. (2007) also report that ovarian cancer cell lines with higher levels of acetylated cortactin migrated faster than cell lines with lower levels of acetylated cortactin.

The paper by Zhang et al. (2007) provides a clear explanation for how acetylation regulates the activity of

cortactin. Nonetheless, there is much to learn about the acetylation and deacetylation of cortactin. Is acetylation simply used to turn off cortactin's actin activity or does it play a role in cycles of actin at sites of membrane remodeling? The recruitment of HDAC6 to cortical sites suggests at least that cortactin deacetylation is locally controlled. How is acetylation-deacetylation of cortactin regulated? The involvement of the small GTPase Rac is one possibility, but phosphorylation may be another. Coupling between phosphorylation and acetylation of histones has been observed. Key to the future understanding of cortactin acetylation will be the identification of the cortactin acetyltransferase. This study (Zhang et al., 2007) and earlier work on the tubulin acetyltransferase (Maruta et al., 1986), suggest that these cytosolic acetyltransferase(s) may differ from those involved in histone acetylation.

Another important implication of this paper (Zhang et al., 2007) is that it indicates that the role of HDAC6 in cell migration is not limited to microtubule acetylation-deacetylation. Instead, HDAC6, and probably SIRT2, appear to regulate both the microtubule and actin cytoskeletons. Thus, studies using drugs which inhibit HDAC6 will now need to account for their affects on both cytoskeletal elements. It will be interesting to test whether cortactin deacetylation is affected by the HDAC6 inhibitor tubacin, which has been suggested to be specific for tubulin deacetylation (Haggarty et al., 2003).

The work of Zhang et al. (2007) has added cortactin to the ever-growing HDAC6-pack and it is very likely that the number of HDAC6-regulated cytoplasmic proteins will continue to grow. A recent proteomic survey of acetylated proteins identified over 180 new lysine-acetylated proteins, including actin itself (Kim et al., 2006). It will be important to determine which of these proteins are deacetylated by HDAC6 to better understand how this remarkable enzyme regulates complex cellular processes such as cell migration.

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14-3-3 Proteins in Plant Brassinosteroid Signaling

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Brassinosteroid (BR) signaling requires the BIN2 kinase-promoted interaction of 14-3-3 proteins with the transcriptional regulators BZR1 and BZR2, which are subsequently redistributed to the cytoplasm by BRs. In this issue of *Developmental Cell*, Gampala et al. show that this redistribution may fine-tune BR responses and serve to crosstalk with other signaling pathways.

14-3-3 proteins received their somewhat ineffable name from being in fraction 14 of an ion-exchange column and being in fraction 3.3 after starch gel electrophoresis of brain extracts (Moore and Perez, 1967), and they have since been found in all eukaryotes. 14-3-3s function as dimeric proteins (Figure 1) in which each monomer is capable of binding to common peptide motifs found in target proteins. The target motifs can undergo serine phosphorylation that subsequently changes the affinity of the target proteins for binding to 14-3-3s. 14-3-3s themselves can also be phosphorylated by (for example) PKC isoforms, and this prevents interaction with target proteins (Aitken, 2006). The physiological functions of 14-3-3s are quite diverse and range from transcriptional regulation through interaction with transcription factors to activation of membrane proteins such as ATPases, and they are often implicated in protein translocation between the cytosol and the nucleus (Aitken, 2006). In *Arabidopsis* at least 12 different 14-3-3 proteins have been identified that appear to have isoform-specific subcellular