# Comparative Phosphoproteomic Analysis of Neonatal and Adult Murine Brain

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Developmental processes are governed by diverse regulatory mechanisms including a suite of signaling pathways employing reversible phosphorylation. With the advent of large-scale phosphoproteomics it is now possible to identify thousands of phosphorylation sites from tissues at distinct developmental stages. We describe here the identification of over 6,000 non-redundant phosphorylation sites from neonatal murine brain. When compared to nearly three times the number of phosphorylation sites identified from three-week-old murine brain, remarkably one-third of the neonatal sites were unique. This fraction only dropped to one-quarter when allowing the site to stray plus or minus 15 residues. Using quantitative mass spectrometry we characterized a novel phosphorylation site (Ser265) identified uniquely in the neonatal brain on Doublecortin (Dcx), a protein essential for proper mammalian brain development. While the relative levels of Dcx and phospho-Ser265 Dcx between embryonic and neonatal brain were similar, their levels fell precipitously by postnatal day 21, as did phospho-Ser297, a site required for proper neuronal migration. Both sites lie near the microtubule-binding domain and may provide functionally similar regulation via different kinases. These data provide evidence for considerable change in the profiles of developmentally-regulated phosphoproteomes. Toward a more global characterization of the changes of proteomes and phosphoproteomes across brain development, we also present emerging data from a quantitative proteomics experiment comparing embryonic and adult murine brain.