Analysis of phosphorylation of the nuclear chaperona nucleoplasmin

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Multisite phosphorylation of proteins is a widely spread mechanism aimed to regulate protein function and protein-protein interactions. Nucleoplasmin (NP), the first molecular chaperone ever described, is a pentameric protein built of identical 200 amino acid subunits each organized in two domains. The core domain, corresponding to the 120 N-terminal amino acids, is responsible for oligomerization and confers the protein an extreme thermal stability (Hierro, 2002). The tail domain, which comprises the remaining 80 residues, harbours a segment rich in negatively charged residues ("poli-glu") and a nuclear localization signal (NLS), and it is thought to adopt a natively disordered conformation (Hierro, 2001). NP is involved in nucleosome assembly, chromatin decondensation at fertilization and apoptosis (Leno, 1996; Lu, 2005). To carry out these activities NP has to interact with different types of histones. As a means to do that, NP removes sperm nuclear basic proteins, replacing them with histones. Binding of NP to basic proteins has been commonly assigned to the negatively charged poly-glu tract, which contains 20 aspartic and glutamic acid residues among the C-terminal 30 residues. However, it has been proved that the phosphorylated core domain, lacking this poly-glu tract, is also able to decondesate chromatin by its own, suggesting that the poly-glu tract is not the only region involved in NP activity and ligand binding (Bañuelos, 2003).

With the aim of identifying the phosphorylation sites present in NP isolated from eggs of *Xenopus laevis*, a strategy that combines TiO_2 enrichment of phosphorylated peptides and mass spectrometry was used. We identified eight phosphorylated sites. Our results show that among the eight phosphoryl groups experimentally detected, four are located at the flexible N terminus, and the rest are found at the tail domain, flanking the nuclear localization signal. These results indicate that phosphorylation of both protein domains is required for NP to efficiently extract linker-type histones from chromatin (Bañuelos, 2007).

References

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