Structural biology and regulation of the classical nuclear import pathway

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Proteins are translated in the cytoplasm, but many need to access the nucleus to perform their functions. Nuclear transport factors recognize nuclear targeting signals on these cargo proteins, and the best-characterized are the classical nuclear localization signals (cNLSs), recognized by the adaptor protein importin-α.Importin-α in turn binds to importin-β, and the trimeric complex is transported into the nucleus through the nuclear pore complex. The key regulator of this transport cycle is the small GTPase Ran.We have used structural biology to elucidate the molecular basis of nuclear protein import.Through determining a number of structures of cNLS:importin-α complexes, we have defined the molecular basis of recognition of typical and atypical cNLS. Post-translational modifications such as phosphorylation provide further regulation to the classical nuclear import pathway, for example the phosphorylation by the cyclin-dependent kinase 1 (Cdk1) adjacent to nuclear localization signals (NLSs). We found that numerous human proteins have Cdk1-dependent phosphorylation sites adjacent to their NLSs. These proteins are involved in key regulatory events of DNA repair, epigenetics, or RNA editing and splicing. Cell-cycle dependent events of genome editing and gene expression profiling may therefore be controlled by nucleocytoplasmic trafficking. We selected a number of proteins and analyzed how point mutations, expected to modify the phosphorylation ability of the NLS segments, perturb nucleocytoplasmic localization. In each case, we found that mutations mimicking hyper-phosphorylation abolished nuclear import processes. We performed a video microscopy-based kinetic analysis to obtain information on cell-cycle dynamics on a model protein, dUTPase, an enzyme essential for genomic integrity. We show that the NLS-adjacent phosphorylation by Cdk1 of human dUTPase results in dynamic cell cycle-dependent distribution of the protein. Non-phosphorylatable mutants have drastically altered protein re-import characteristics into the nucleus during the G1 phase. The structure of phospho-mimic dUTPaseNLS:importin-α complex shows how the introduction of negative charge disrupts the interactions of the NLS with the nuclear transport factor. Our results suggest a dynamic Cdk1-driven mechanism of regulation of the nuclear proteome composition during the cell cycle.