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Casein kinase II associates with and phosphorylates the nucleosome assembly protein (NAP1) in *Drosophila melanogaster*

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The nucleosome assembly protein-1, or NAP1, was originally identified in HeLa cells as a factor which facilitates the *in vitro* assembly of nucleosomes from naked plasmid DNA and core histone proteins. However, in yeast cells it was shown that NAP1 is required for the ability of Clb2, a major cyclin B of the budding yeast, to induce specific mitotic events. Previous findings of our laboratory have revealed that in *Drosophila* NAP1 can be associated with cyclin B and bound to the components of the spindle during mitosis.
To gain insights into the NAP1 function, I have used molecular and biochemical methods to identify proteins that bind specifically to *Drosophila* NAP1. This approach has led me to identify a kinase associated with NAP1 and able to specifically phosphorylate this protein. By using an in-gel kinase assay I can show that the kinase phosphorylating NAP1 displays a molecular mass of 38-kDa. Purification of this protein and peptide microsequencing revealed that it corresponds to the catalytic alpha subunit of casein kinase II (CKII).
Use of a „back“ phosphorylation procedure involving dephosphorylation of the native NAP1 by protein phosphatase PP2A prior to *in vitro* phospho-labelling revealed that the phosphatase treatment markedly increases the subsequent phosphorylation of NAP1 by CKII, suggesting that the CKII sites present in NAP1 are phosphorylated *in vivo*. Furthermore, immunostaining analysis showed that in *Drosophila* embryos CKII is localized on the spindle during mitosis. Therefore, the interaction of NAP1 with CKII suggests that CKII phosphorylation may play a crucial role in the dynamics of the spindle through its association with NAP1.
With the help of a series of truncated NAP1 recombinant proteins and synthetic peptides, I was able to determine two major CKII phosphorylation sites in NAP1 at residues Thr-120 and Ser-284, and one minor site at residue Ser-118. In addition, I show that two distinct regions of NAP1 can bind to the alpha subunit of CKII. Interestingly, the residues Ser-118 and Thr-120 are located within one of two PEST domains identified in NAP1, while the residue Ser-284 flanks the nuclear localization sequence. The location of the CKII phosphorylation may regulate the rate of NAP1 turnover by triggering a PEST-mediated mechanism of protein degradation, and may also control the translocation of NAP1 between the cytoplasm and the nucleus.