# The building of a centromeric nucleosome: Biochemical analysis and comparison of Cse4p, histone H3 and Cse4-286p to define the unique assembly pathway of a Cse4p nucleosome

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## Abstract

Nucleosomes are the most fundamental unit of chromatin structure, which is composed of ~～145 bp of DNA wrapped around an octamer of core histones H2A, H2B, H3 and H4. Chromatin structure plays a role in many cellular processes and the eukaryotic cell has devised many different ways to alter the structure of the nucleosome as part of the cellular mechanisms that regulate important processes that occur in the cell. Some of these processes include modification events such as DNA methylation, and histone protein modifications, and in some cases includes the incorporation of two copies of a specialized variant histone into each nucleosome to create a specialized nucleosome at the centromeres. ^ CenH3s represent a family of variant histone proteins that function to replace histone H3 in specialized nucleosomes found only at the centromere. So far CenH3 proteins have been identified at every eukaryotic centromere tested and these proteins are essential. Due to the relative simplicity of its centromere and associated kinetochore proteins, the *S. cerevisiae* CenH3 protein, Cse4p, was first characterized genetically. Cse4p contains a histone fold domain that is 60% identical to the HFD of histone H3, as well as an N-terminus that is unique. It was recently discovered that the localization of Cse4p to the centromere is dependant only on the HFD of the protein and not on the function of the N-terminus. ^ All current evidence supports the idea that Cse4p interacts with histone H4 and the other core histone proteins at the centromere locus, in the absence of histone H3. It is still not known how very low levels of Cse4 proteins can effectively compete with the much more abundant histone H3 proteins to bind to histone H4 and localize to the centromere. I expressed the recombinant Cse4p, histone H3, histone H4 and a cse4 mutant protein (cse4-286p) and purified these proteins in order to compare the biophysical characteristics of Cse4p and the Cse4p/H4 complex with those of histone H3 and the H3/H4 complex. Results of these studies indicate that Cse4p follows an assembly pathway that is distinct from the histone H3 pathway. Mutations that interfere with the Cse4p assembly pathway cause cell death. ^ To determine if pathways similar to the proposed Cse4p-specific assembly pathway occur that involve the CenH3 proteins from other species, I searched several public genomic databases and compiled 160 distinct CenH3 HFD sequences. The newly identified CenH3 sequences were used to generate a multiple sequence alignment (MSA) to look for a functional connection between the CenH3 HFD primary sequences and the homodimerization of Cse4p, which is mediated through the 4-helix bundle. Statistical coupling analysis (SCA) of the MSA reveals that amino acids that are involved in making DNA contacts exhibit covariance with amino acids that probably play a role in homodimerization. These results suggest a connection between homodimerization of Cse4p and DNA-protein interactions. Another

cluster of covarying Cse4p residues were identified, which most likely play a role in Cse4p interactions with histone H4. One amino acid residue in this cluster was previously identified in budding yeast cells to cause a temperature sensitive phenotype when mutated, which was rescued by over-expression of the histone H4 protein. ^ This proposed new centromeric nucleosome assembly pathway provides an elegant, testable model to determine how CenH3 proteins compete with histone H3 to interact with histone H4. ^

## Subject Area

Molecular biology|Biochemistry

## Recommended Citation