A flexible bipartite coiled coil structure is required for the interaction of Hexim1 with the P-TEFB subunit cyclin T1

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Transcription elongation is regulated by the cellular protein Hexim1, which inhibits phosphorylation of RNA polymerase II by interacting with the positive transcription elongation factor P-TEFb. Hexim1 binds directly to Cyclin T1 of P-TEFb with its coiled coil domain that is subdivided into a highly polar N-terminal segment containing nonconservative residues in the dimer interface and a C-terminal segment with an evolutionarily conserved sequence composition. Here we show that the noncanonical sequence composition of the first coiled coil segment is required for the interaction with Cyclin T1 while the second segment keeps the Cyclin T-binding domain dimeric upon binding. Both coiled coil segments exhibit distinct melting points as shown by heat denaturation experiments using circular dichroism spectroscopy. Deletion of the central stammer motif (Delta316-318) leads to a single denaturation reaction, suggesting formation of a continuous coiled coil. Mutation of noncanonical coiled coil residues K284 and Y291 to valines in the dimer interface of the first segment only slightly increases its stability. Concomitantly, deletion of the stammer but not the double point mutation led to a reduced affinity for Cyclin T1 as shown by isothermal titration calorimetry. Moreover, Cyclin T1 bound Hexim1 with a 1:2 stoichiometry, whereas truncation of the C-terminal coiled coil led to formation of an equimolar complex. These observations suggest that binding to Cyclin T1 induces an asymmetry or sterical hindrance in the first coiled coil segment of dimeric Hexim1 that disallows formation of a 2:2 complex as further supported by analytical ultracentrifugation and cross-linking experiments.

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