A Kinase Subunit of the Human Mediator Complex, CDK8, Positively Regulates Transcriptional Activation

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The human TRAP/Mediator and its related complexes mediate transcription through regulatory factors. To further understand the structural and functional diversity of these complexes, we established three HeLa cell lines each expressing one of three epitope-tagged human TRAP/Mediator subunits, MED6, MED7, and CDK8, and isolated the complexes in which these subunits were contained by affinity and HPLC-gel filtration chromatography. The largest complexes from each cell line had a molecular mass of 1.5 MDa and possessed almost identical subunit compositions; we designated these complexes TMLC1 (TRAP/Mediator-like complex 1). Two potential subcomplexes were additionally observed: a 1-MDa complex from the CDK8-cell line (TMLC2) and a 600-kDa complex from the MED6-cell line (TMLC3). All three complexes regulated transcription *in vitro*; TMLC1 and TMLC3 augmented transcriptional activation, whereas TMLC2 repressed it. TMLC1 and TMLC2 phosphorylated RNA polymerase II, but TMLC3 did not. Furthermore, TMLC1 predominantly interacted with the general transcription factors TFIIE, TFIIF, and TFIIH, which function during transcription initiation and the transition to elongation. In a final experiment, knockdown of CDK8 using RNA interference prevented transcriptional activation by Gal4-VP16 in a luciferase-assay. We have demonstrated that the introduction of siRNA-resistant CDK8 expression plasmid into the CDK8 knock down cells counteracted the repressive effects in transcription and have proven that this effect is caused by CDK8. Intriguingly, CDK8-knock down also efficiently reduced CTD phosphorylation of Pol II. This, together with the effect of TMLC1 on transcription *in vitro*, suggests that CDK8 play positive roles in transcriptional activation and that CTD phosphorylation of Pol II is intimately related to its function.