

Discovery of Mutually Exclusive Mediator-Kinase Module Protein Complexes

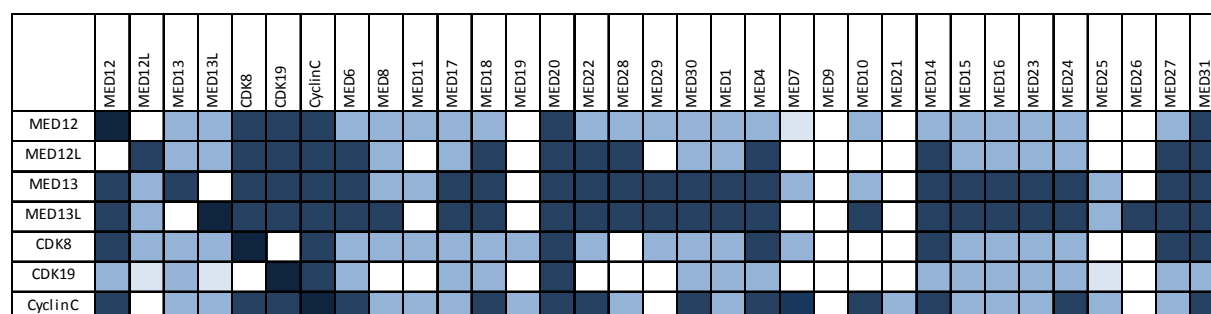
The understanding of protein complex assembly and mapping of protein interactions has rapidly grown in recent years due to significant advances in mass spectrometry. Combined with selective enrichment tools we can now probe with high degree of selectivity and sensitivity protein/protein interactions and the individual networks they represent within a protein complex. Here we have used HaloTag fusions combined with MS Bioworks IP-works service to isolate Mediator:Kinase module complexes. This strategy enables visualization with previously unparalleled resolution of the specific interactions of the subcomplex with core Mediator transcriptional activator complex as a whole.

Methods

Members of the Mediator kinase module were genetically fused to the HaloTag and transiently expressed as protein fusions in HEK293 cells. The respective Mediator complexes were isolated directly from lysates using a HaloTag pull-down protocol. Interacting protein partners were analyzed for composition using nanoscale LC-MS/MS coupled with an LTQ Orbitrap Velos (Thermo). Database searching of peptide fragmentation data was performed with Mascot (Matrix Science) and data validation and visualization was performed using Scaffold (Proteome Software) with quantitation and normalization based on spectral counts and normalized spectral abundance factors (NSAF). Each pull-down experiment was performed in triplicate.

Results

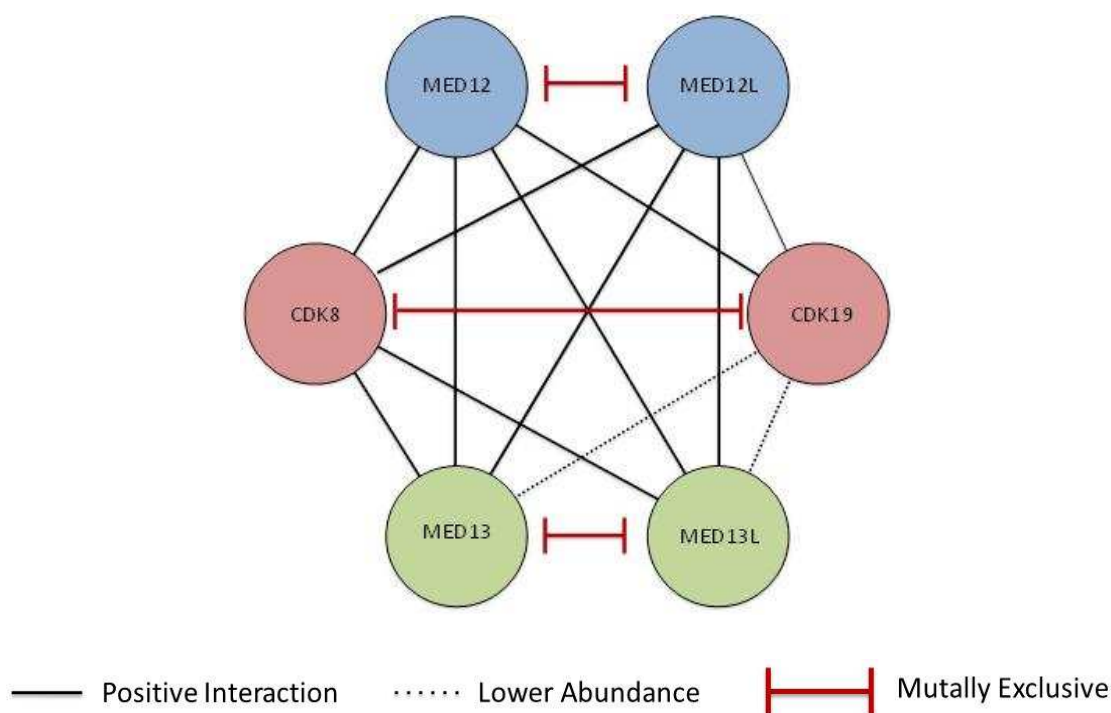
LC-MS/MS data from tagged kinase module subunits MED12, MED13, CDK8 and Cyclin-C and their paralogues MED12L, MED13L and CDK19 were used to generate the heat below. Here the relationship between the tagged proteins and known members of the Mediator complex can be visualized.



NSAF Values	1-1E-04	1-1E-03	1-1E-02
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Summary

Using the strategy described above it was possible to fully characterize the protein-protein interactions between the Mediator complex and the kinase module. This revealed Kinase module subunits MED12, MED13 and CDK8 are mutually exclusive with their paralogues MED12L, MED13L and CDK19 when bound to core Mediator. Cyclin-C is consistently observed with the other six kinase module subunits. This data suggests different regulatory roles of Mediator-Kinase module complexes. The data are summarized in the figure below.



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