Discovery of Mutually Exclusive Mediator-Kinase Module Protein Complexes

Richard Jones, Michael Ford, Ravi Amunugama, David Allen
MS Bioworks, 3950 Varsity Drive, Ann Arbor, MI 48108
Marie Schwinn, Jacqui Mendez, Nancy Murphy, Hélène Benink, Danette L. Daniels, Marjeta Urh
Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711

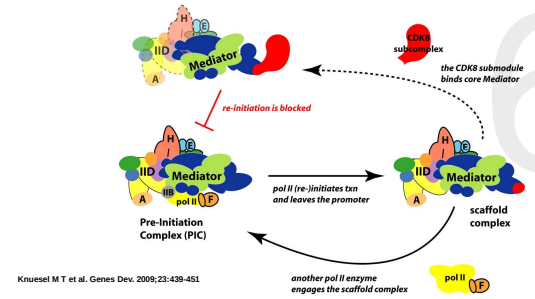
MS Bioworks Biological Mass Spectrometry Solutions

Promega

1. Abstract and introduction

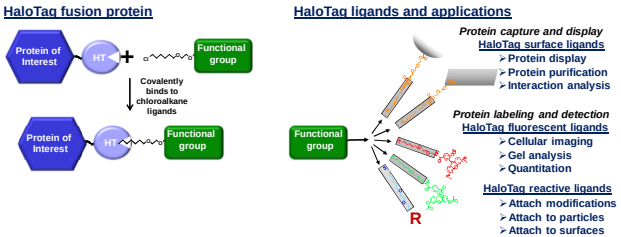
The understanding of protein complex assembly and mapping of protein interactions has rapidly grown in recent years due to significant advances in mass spectrometry. Combined with selective enrichment tools we can now probe with high degree of selectivity and sensitivity protein/protein interactions and the individual networks they represent within a protein complex. Mediator is a 1.2MDa macromolecular complex which functions as an important transcriptional co-activator with RNA polymerase. Here we have used HaloTag fusions 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.

2. Mediator Transcriptional Co-activator and Design



- Mediator exists in two forms: Core Mediator and Mediator + Kinase Module.
- Place HaloTag on all Kinase Module components: MED12, MED12L, MED13, MED13L, CDK8, CDK19 and Cyclin C.

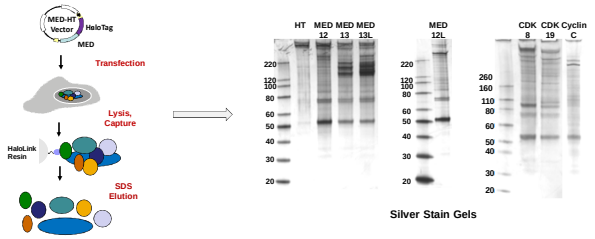
3. HaloTag technology



- HaloTag (HT) is a genetically encoded 33kDa protein fusion tag.
- Engineered to covalently bind various ligands, imparting multi-functionality.

4. Mediator Kinase Module Isolation

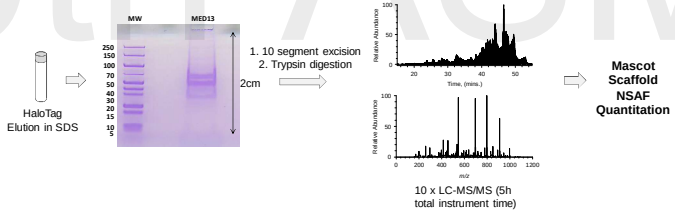
Expression and pull-down of Halo-Mediator Kinase module fusions in HEK293:



- Each kinase module is prepared in biological triplicate.
- Significant enrichment over control of all seven kinase module proteins.

5. Mass Spectrometry and Informatics Platform

Canned workflow for IP analysis:



- Complex is eluted in SDS and loaded on a 10% Novex gel. This platform allows a greater tolerance for IP elution buffers than the equivalent solution-based approaches.
- Gel excised into 10 equal segments, each digested with trypsin and analyzed on a 30 min gradient (Orbitrap Velos Pro). This is 5h total instrument time per complex.
- Data are searched in Mascot and processed in Scaffold at <1% protein FDR.
- Spectral counts are converted to Normalized Spectral Abundance Factors (NSAF) and a mean value calculated for triplicate runs and quantitatively compared to control lanes.
- Significant interactors are reported: binary differences or four-fold higher based on mean NSAF.

Contact Information

Web: www.promega.com www.msbioworks.com

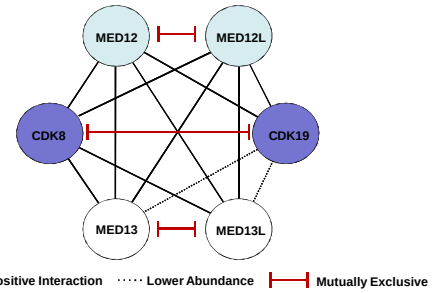
E-mail: info@msbioworks.com

6. NSAF Plot of Mediator and Kinase Module Subunits

	MED12	MED12L	MED13	MED13L	CDK8	CDK19	CyclinC
MED12	SELF		3.6E-03	3.6E-03	1.4E-03	8.3E-04	1.2E-03
MED12L		SELF	5.3E-04	5.9E-04	1.9E-04	7.9E-05	
MED13	1.8E-04	5.5E-04	SELF		4.9E-04	2.9E-04	3.2E-04
MED13L	1.8E-04	2.2E-04		SELF	1.3E-04	4.7E-05	5.3E-04
CDK8	2.0E-03	2.5E-03	2.6E-03	3.8E-03	SELF		5.3E-03
CDK19	1.5E-03	1.2E-03	1.2E-03	2.3E-03		SELF	4.3E-03
CyclinC	2.4E-03	2.0E-03	3.9E-03	4.6E-03	3.9E-03	2.8E-03	SELF
MED6	4.0E-04	1.2E-03	2.7E-03	4.1E-03	7.2E-04	4.0E-04	1.1E-03
MED8	2.6E-04	9.7E-04	9.7E-04	2.4E-03	4.2E-04		9.6E-04
MED11	5.5E-04		5.5E-04		2.2E-04		8.9E-04
MED17	7.0E-04	9.1E-04	3.6E-03	4.0E-03	7.1E-04	4.9E-04	9.9E-04
MED18	5.1E-04	1.5E-03	3.1E-03	2.7E-03	6.6E-04	1.2E-04	1.6E-03
MED19					2.6E-04		1.2E-04
MED20	1.1E-03	2.4E-03	7.0E-03	5.8E-03	2.0E-03	1.5E-03	2.7E-03
MED22	6.7E-04	1.5E-03	2.2E-03	4.3E-03	7.2E-04		1.9E-03
MED28	5.6E-04	1.2E-03	1.1E-03	1.8E-03			3.4E-04
MED29	2.2E-04		1.2E-03	2.6E-03	5.2E-04		
MED30	8.9E-04	9.3E-04	3.3E-03	3.3E-03	3.1E-04	2.1E-04	1.4E-03
MED1	2.9E-04	9.6E-04	2.6E-03	2.9E-03	4.0E-04	2.7E-04	6.9E-04
MED4	7.3E-04	1.3E-03	3.6E-03	6.5E-03	1.6E-03	4.7E-04	1.0E-03
MED7	6.9E-05		5.3E-04		2.6E-04		1.4E-03
MED9							
MED10	3.5E-04		8.9E-04	2.4E-03			1.0E-03
MED21							8.1E-04
MED14	6.9E-04	1.4E-03	3.3E-03	3.4E-03	1.2E-03	7.8E-04	1.4E-03
MED15	2.6E-04	4.7E-04	2.0E-03	2.7E-03	3.5E-04	2.8E-04	2.3E-04
MED16	4.0E-04	7.5E-04	2.0E-03	1.8E-03	4.3E-04	2.7E-04	6.9E-04
MED23	6.8E-04	9.5E-04	3.2E-03	2.4E-03	9.2E-04	6.6E-04	8.4E-04
MED24	5.3E-04	8.6E-04	3.0E-03	2.2E-03	7.3E-04	5.1E-04	1.0E-03
MED25			7.9E-04	6.9E-04		5.4E-05	2.3E-04
MED26				1.5E-03			
MED27	4.2E-04	1.5E-03	3.8E-03	3.0E-03	1.0E-03	2.4E-04	7.5E-04
MED31	1.7E-03	3.4E-03	3.4E-03	3.6E-03	1.2E-03	8.9E-04	1.9E-03

1.1E-04
1.1E-03
1.1E-02

7. Kinase Module Interaction Map



8. Summary

- We present a robust and sensitive workflow for the identification and quantitation of protein complexes.
- All members of the Mediator Kinase module were pulled down using HaloTag in triplicate and interactions characterized.
- Kinase module subunits MED12, MED13 and CDK8 are mutually exclusive with their paralogues MED12L, MED13L and CDK19 when bound to core Mediator.
- These data suggest different regulatory roles of Mediator-Kinase module complexes.

60th ASMS