DNA Chip을 이용한 Transcriptional Activation Mechanism 분석

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Abstract

Mediator of transcriptional regulation is the evolutionary conserved coactivator complex that plays the central role in the integration and recruitment of diverse regulatory signals and transcription machinery to certain promoters. In yeast, each Mediator subunit is required for transcriptional regulation of a distinct group of genes. In order to decipher the mechanistic roles of Mediator proteins in regulating developmental specific gene expression, we isolated, and analyzed a multiprotein complex containing Drosophila Mediator homologs (dMediator). dMediator interacts with several sequence-specific transcription factors and basal transcription machinery, and is critical for activated transcription in response to diverse transcriptional activators. In order to elucidate the function of Mediator in metazoan development, we isolated mutants of a conserved Mediator subunit, Drosophila *Med6* (*dMed6*). *dMed6* null homozygotes failed to pupate and died in the third larval instar. Larval mitotic cells and most imaginal discs showed severe defects in proliferation, but no apparent morphological defect was observed in other larval tissues. Clonal analysis of *dMed6* mutant cells revealed that *dMed6* is essential for cell viability and proliferation of most adult cell types. Drosophila cDNA microarray, quantitative RT-PCR, and *in situ* expression analyses of developmentally regulated genes in *dMed6* mutants showed that transcriptional activation of a subset of genes involved in neuroblast proliferation in the larval brain were most affected. Our results suggest that *dMed6* is required in most cells for transcriptional regulation of a subset of genes important for cell proliferation and metabolism.

CV

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1999년-2001년: 성균관의대 조교수

1994년-1999년: 삼성생명과학연구소 수석연구원/책임연구원

1992년-1994년: Stanford대 세포학과 Post-doctoral fellow

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1986년: 서울대 미생물학과 석사학위 취득

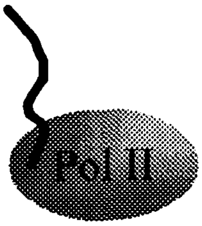
1984년: 서울대 미생물학과 학사학위 취득

RNA polymerase II

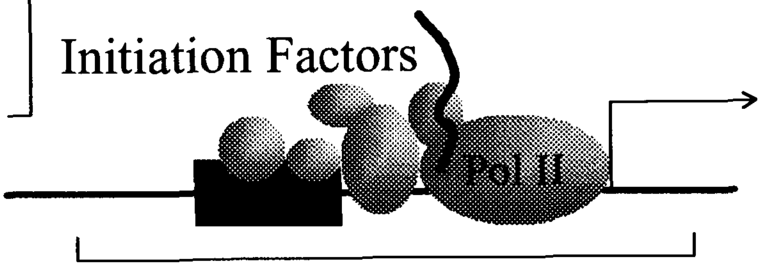
-transcribe all genes in eukaryotes
 except those for some stable RNAs

-15 subunits (12 types): >600 kDa

- unable to recognize a promoter
- unable to initiate transcription
- unable to discharge the products



RNA polymerase II transcription

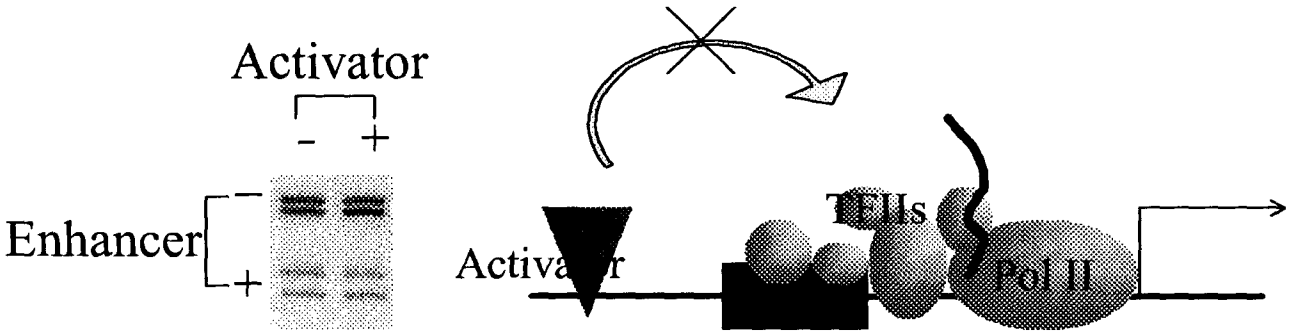


TBP
TFIIB
TFIIE
TFIIF
TFIIH

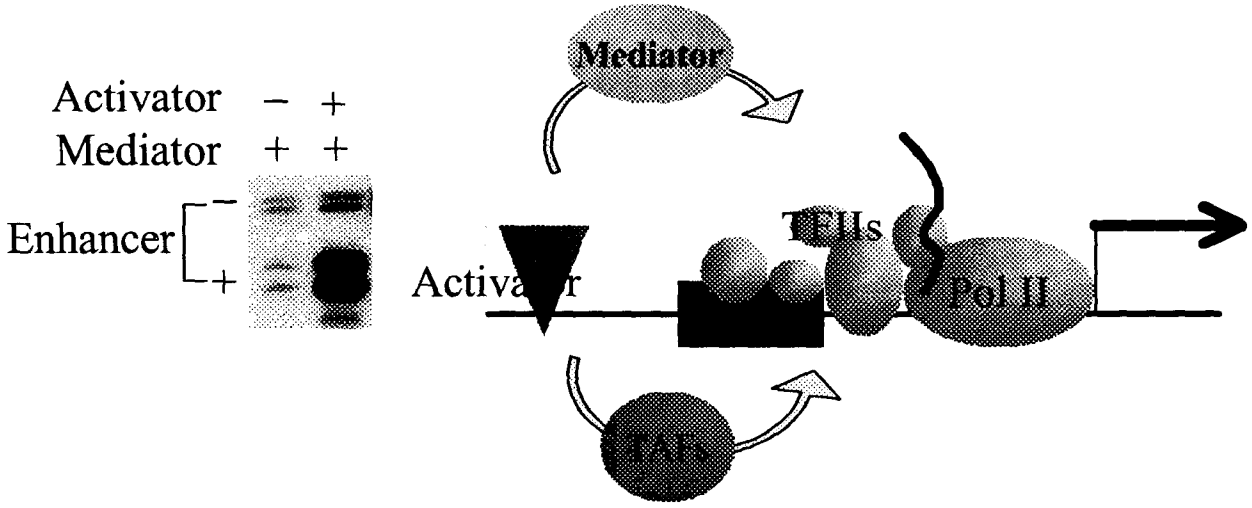
Initiation Factors

Core Promoter

Reconstituted Basal Transcription System does not Respond to Transcriptional Activators.

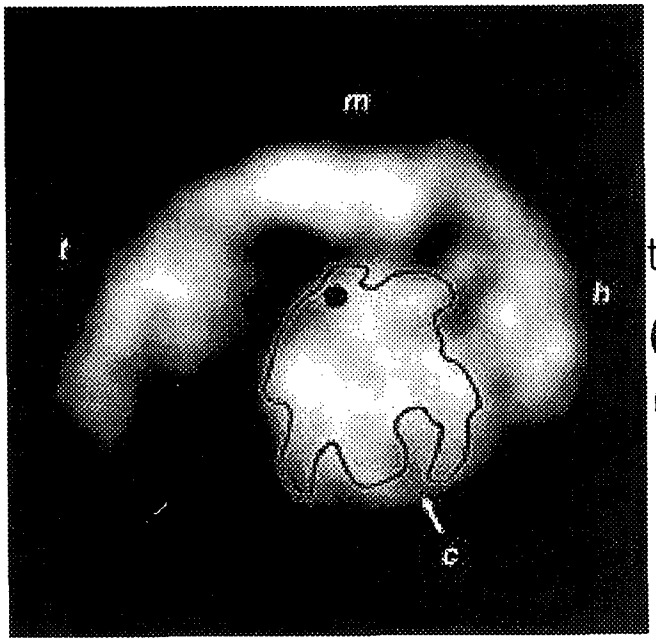


Mediator is Required for Transcriptional Activation.

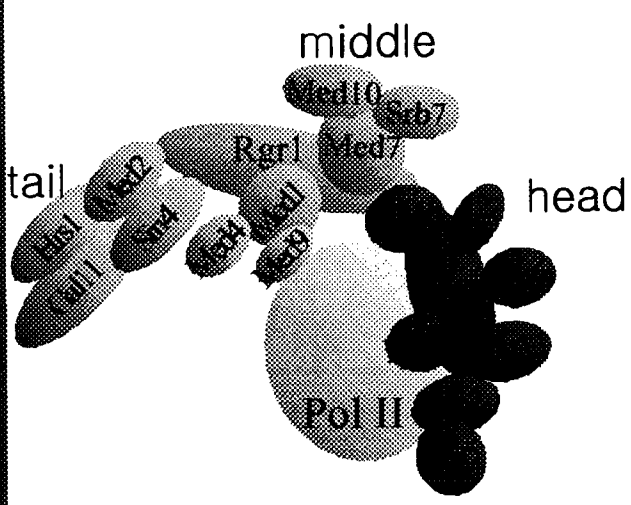


Modular Structure of Mediator Complex

EM Structure

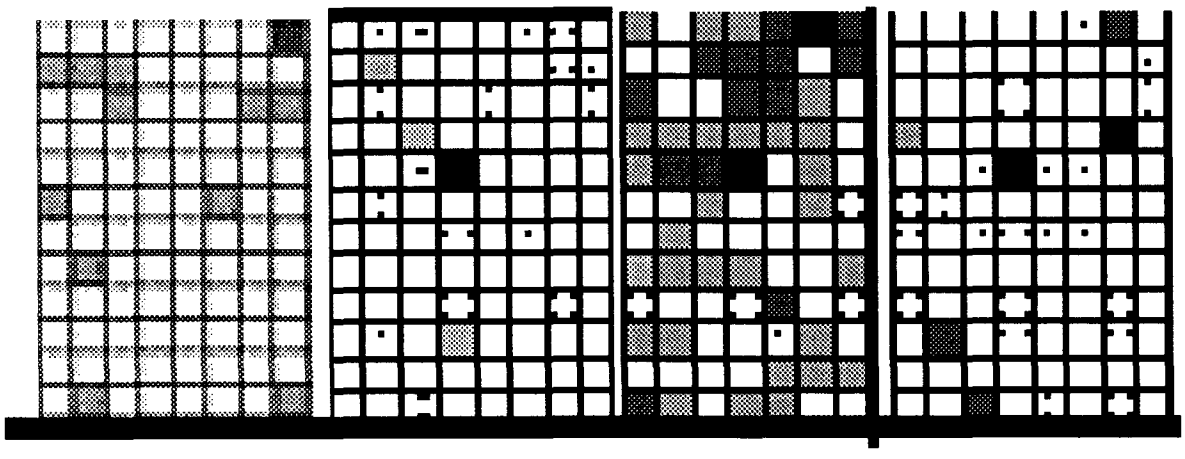


Model

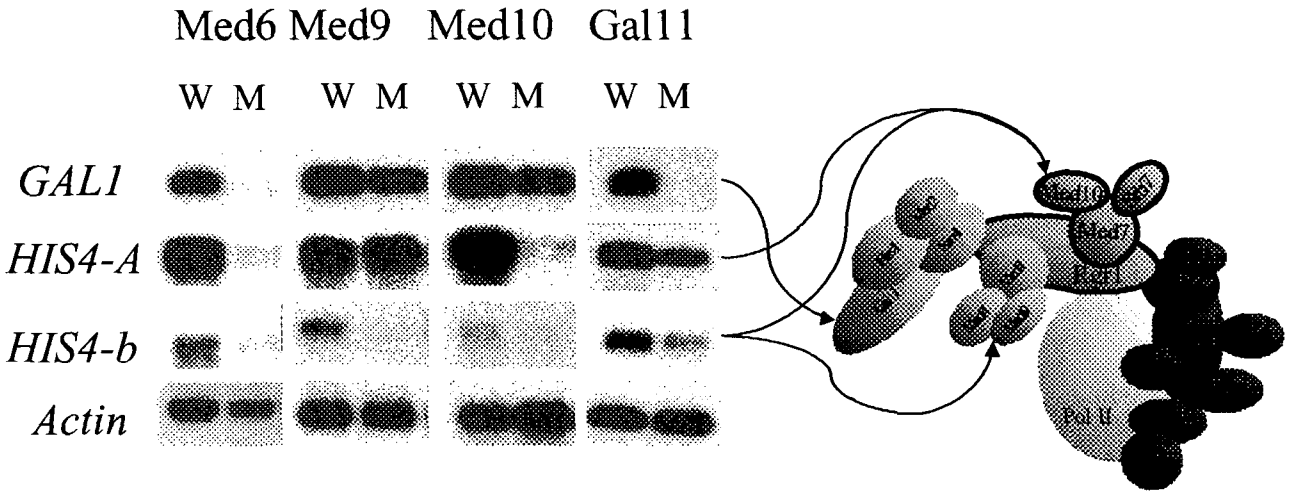


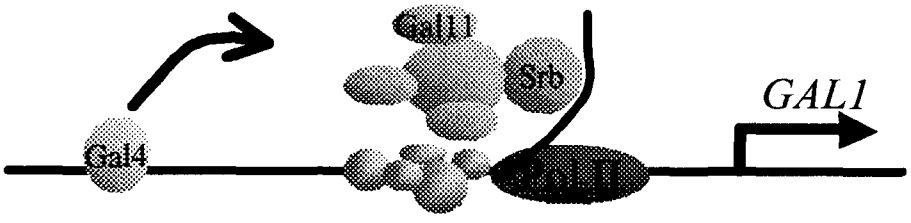
Each Transcription Unit Requires Distinct Set of Mediator Subunits.

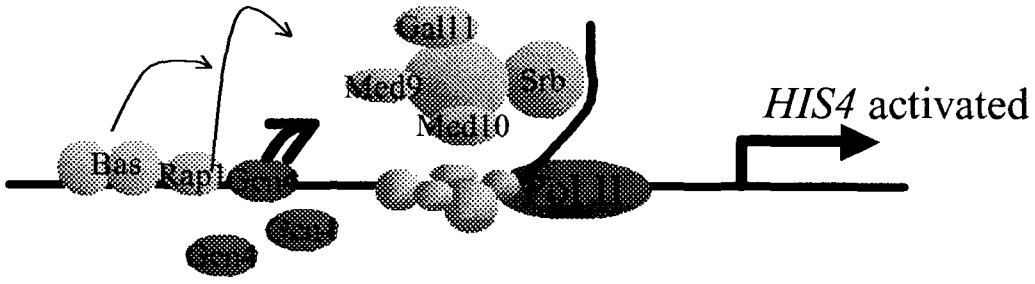
med6ts *med9 ?* *med10ts* *med11ts*



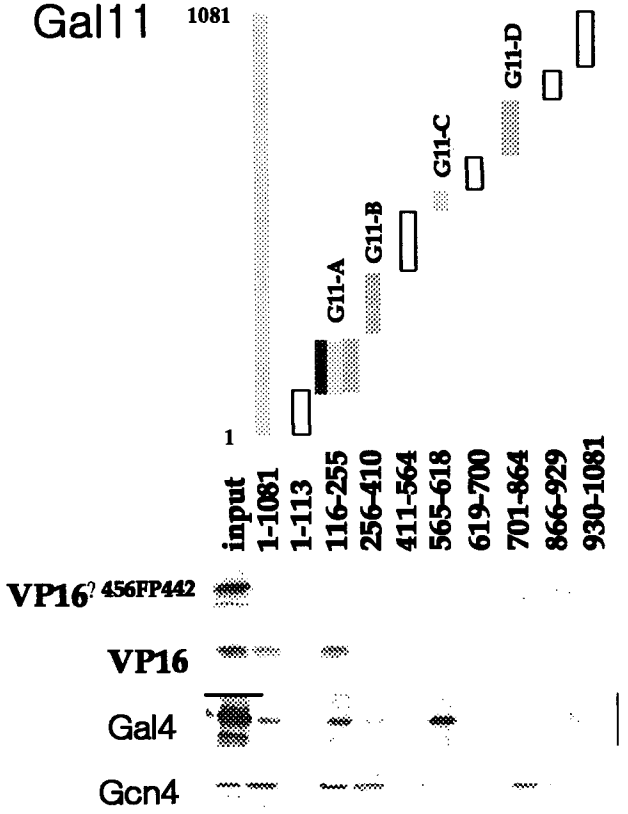
Distinct Transcriptional Defects of Each Mediator Mutant







Activator-specific Binding Regions of Mediator



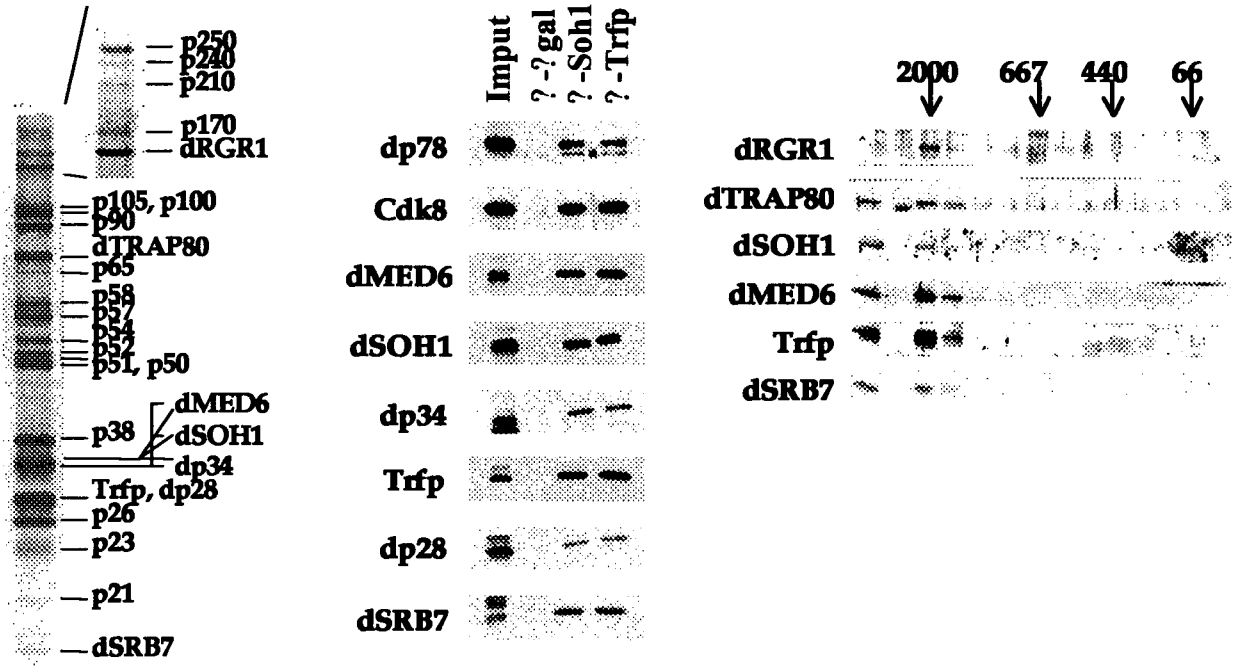
Metazoan Mediator Complex

•Mouse Mediator	R. Kornberg
•Human Mediator (hMediator)	A. Berg
•negative regulator of activated transcription (NAT)	D. Reinberg
•cofactor required for Sp1 activation (CRSP)	R. Tjian
•Srb/Med-containing cofactor complex (SMCC)	R. Roeder
•thyroid hormone receptor-associated protein (TRAP)	R. Roeder
•activator-recruited cofactor (ARC)	R. Tjian
•vitamin D-receptor interacting protein (DRIP)	L. Freedman

•Play a key function in regulating Pol II transcription in vitro
•Show compositional and functional heterogeneity

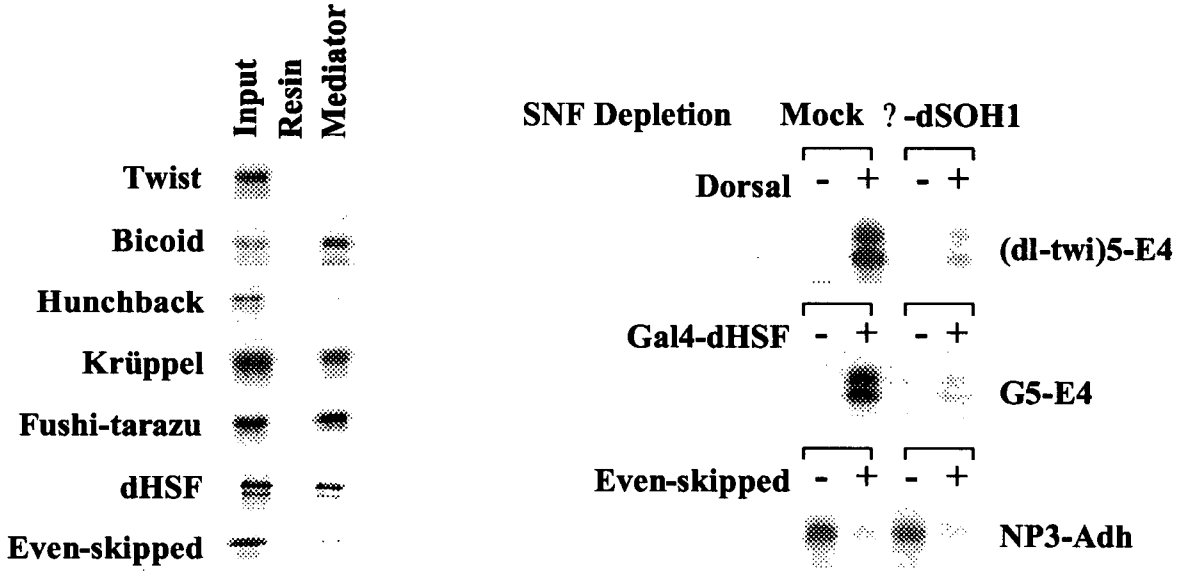
Purification of Drosophila Mediator Complex

Peptide sequencing **IP** **Gel filtration**



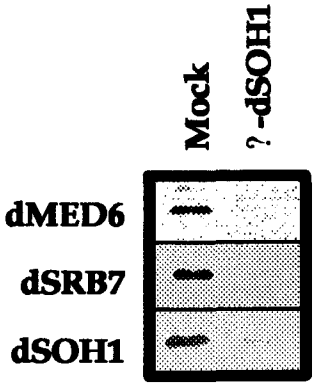
Diverse Activators, but Repressors, interact with Mediator for Transcriptional Regulation

Mediator column binding **In vitro transcription**

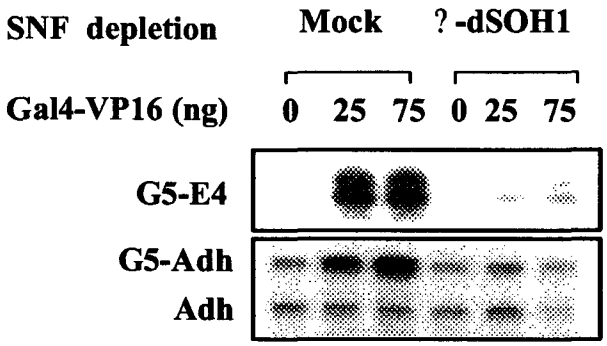


Drosophila Mediator Complex is Required for Transcriptional Activation In Vitro

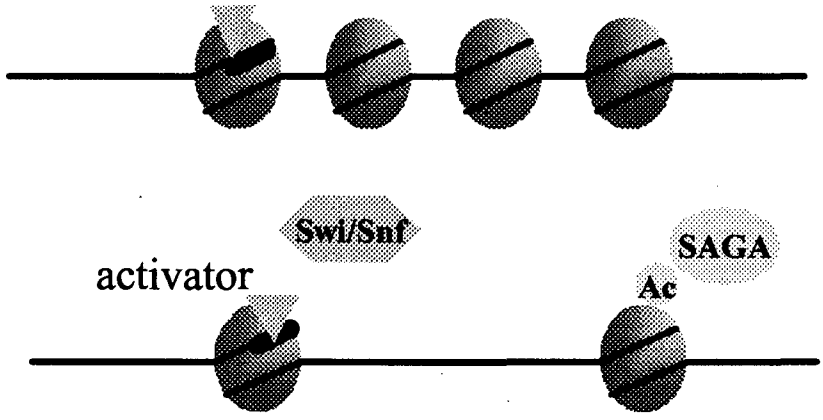
Immunodepletion



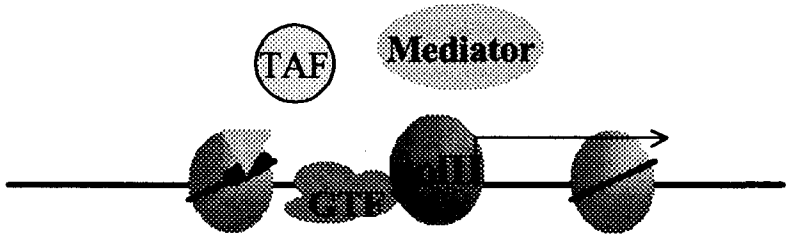
In vitro transcription



Diverse coactivator complexes function at different stages of transcriptional regulation

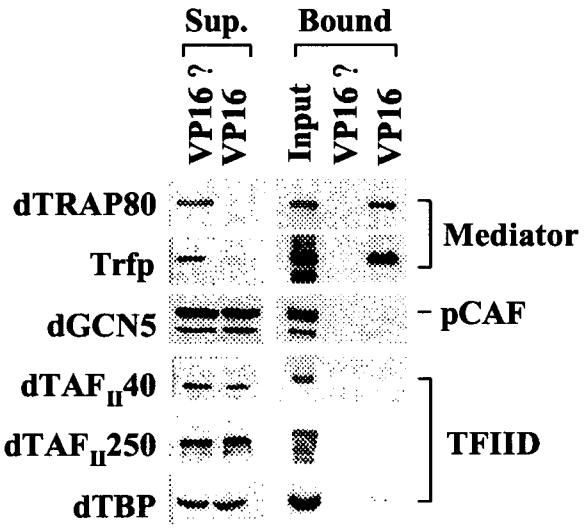


Remodeling
modification

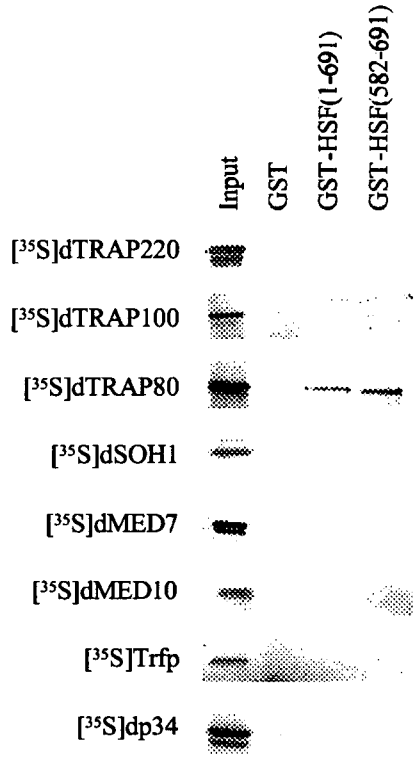


PIC

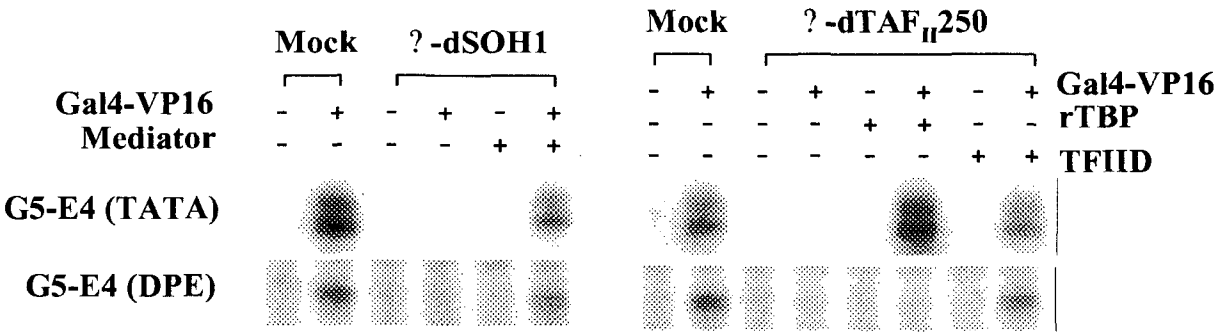
Mediator is the Major Binding Target of Transcriptional Activator



Activator binding site of Drosophila Mediator Complex

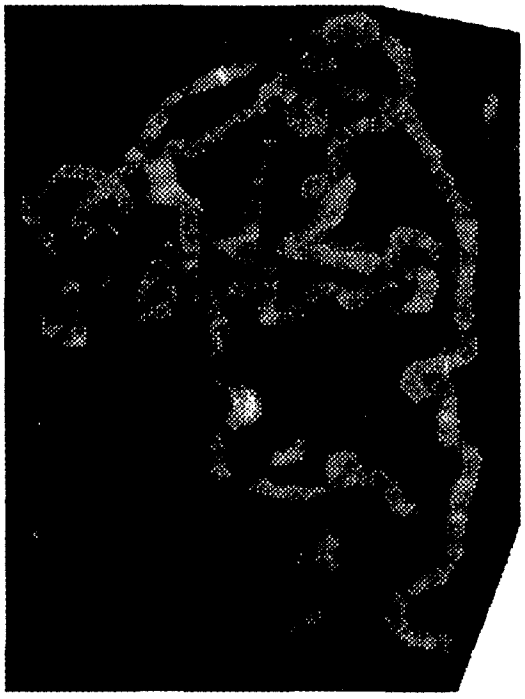


dMediator functions as coactivator at both types of core promoters while TFIID works as Core promoter selectivity factor

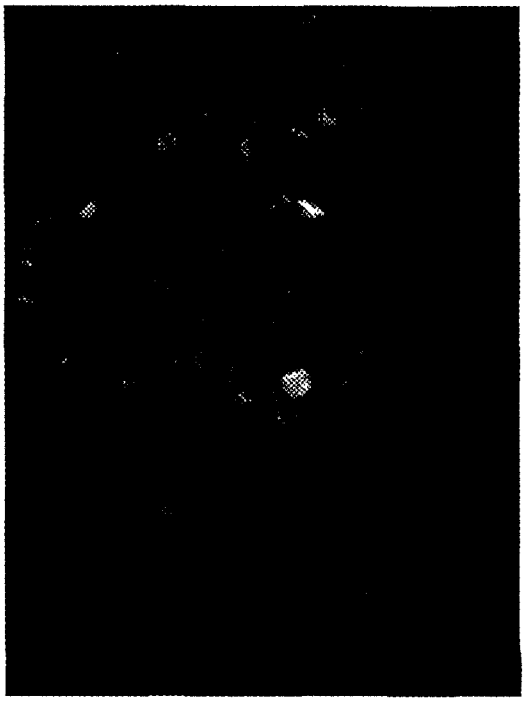


Mediator, but TAF, colocalizes with Heat Shock Factor

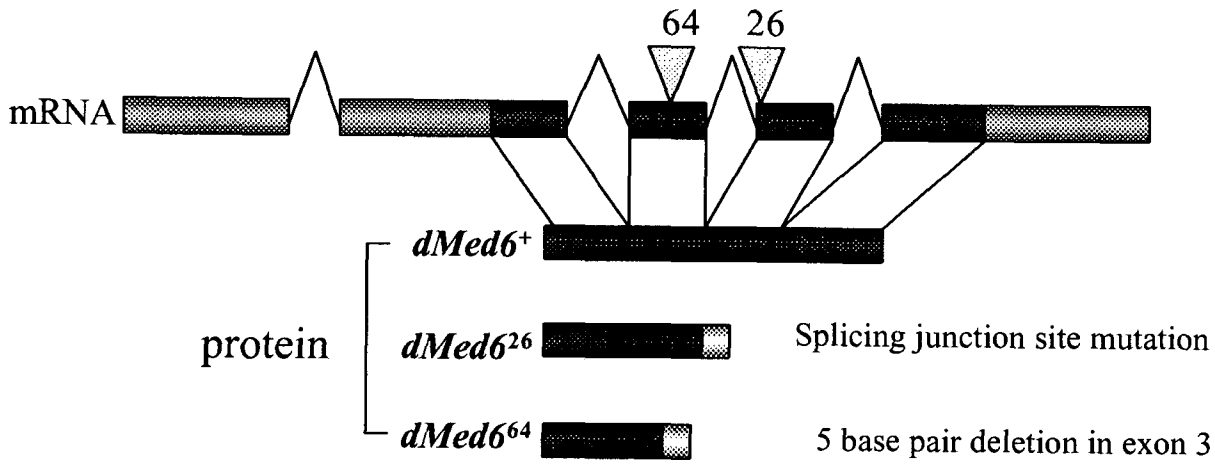
Mediator + HSF



TAFs + HSF

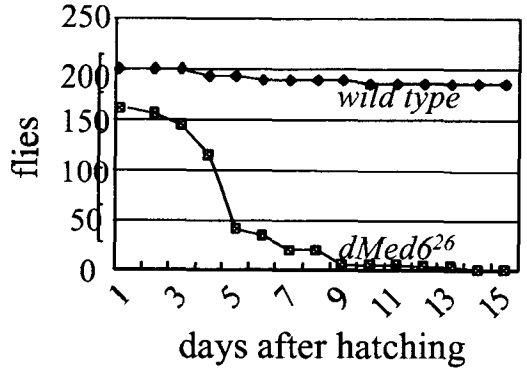


Genomic Structure of *dMed6* Mutant Alleles

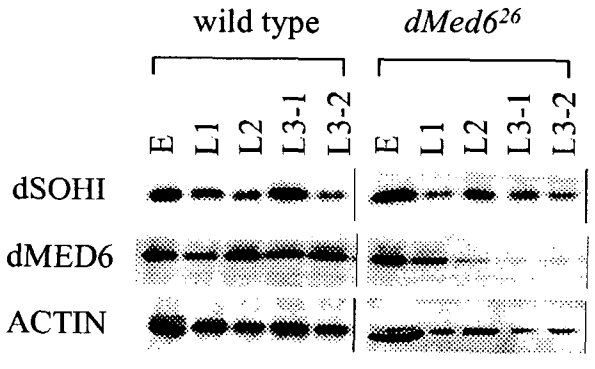


dMed6 is Essential for Larval Development

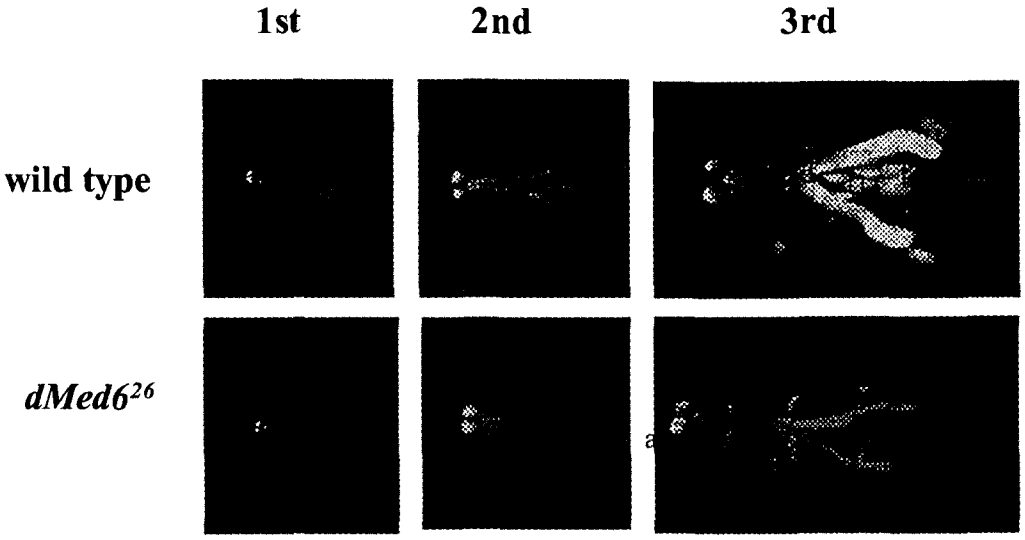
dMed6 null lethal phase



Western analysis of dMed6 mutant

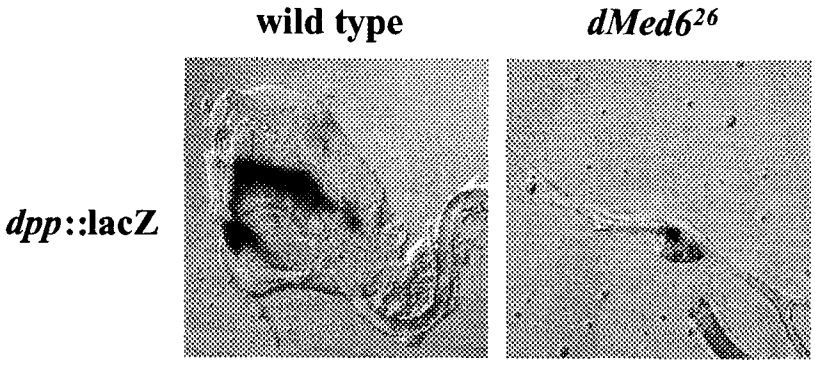


dMed6 mutants showed Defects in Imaginal Disc Development

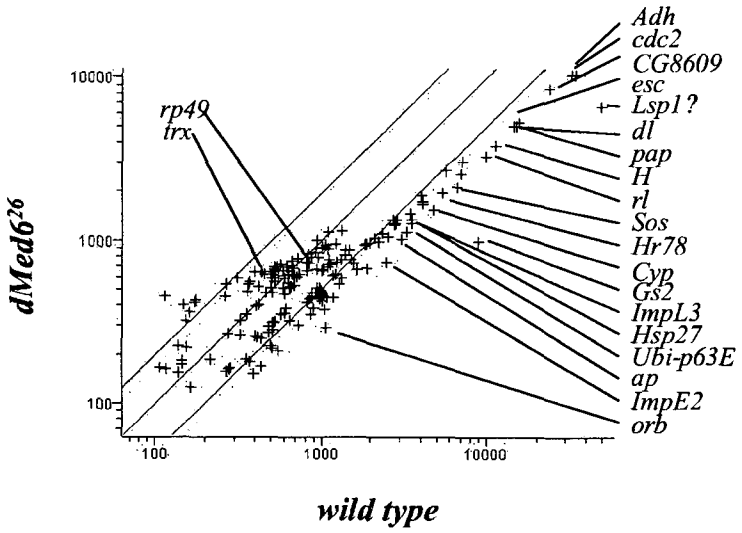


Confocal analysis of *dll*::GFP expression

dMed6 is Required for Imaginal Disc Proliferation

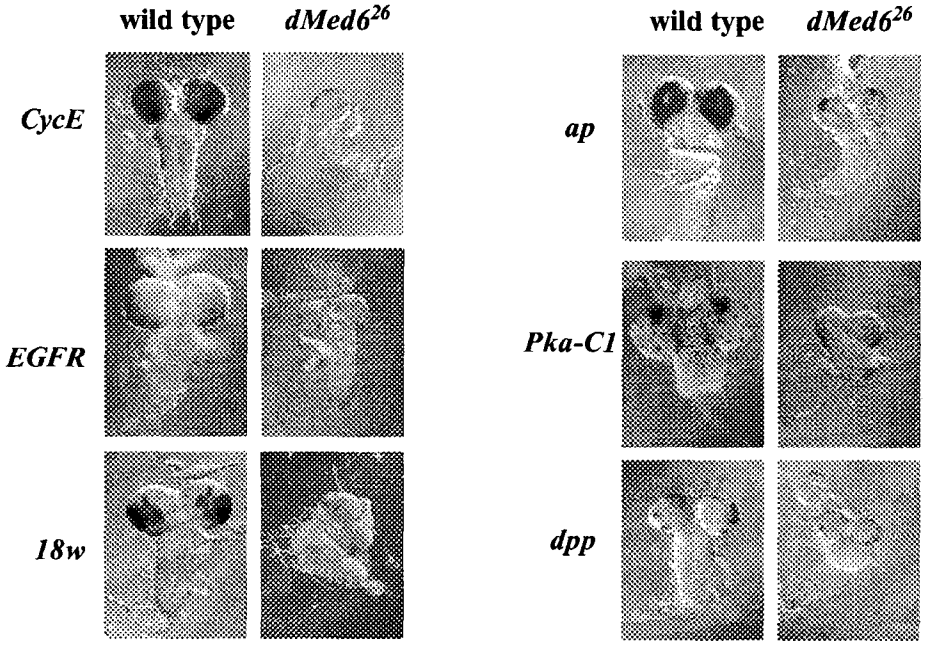


Large Number of Genes Require *dMed6* Function for Transcriptional Activation

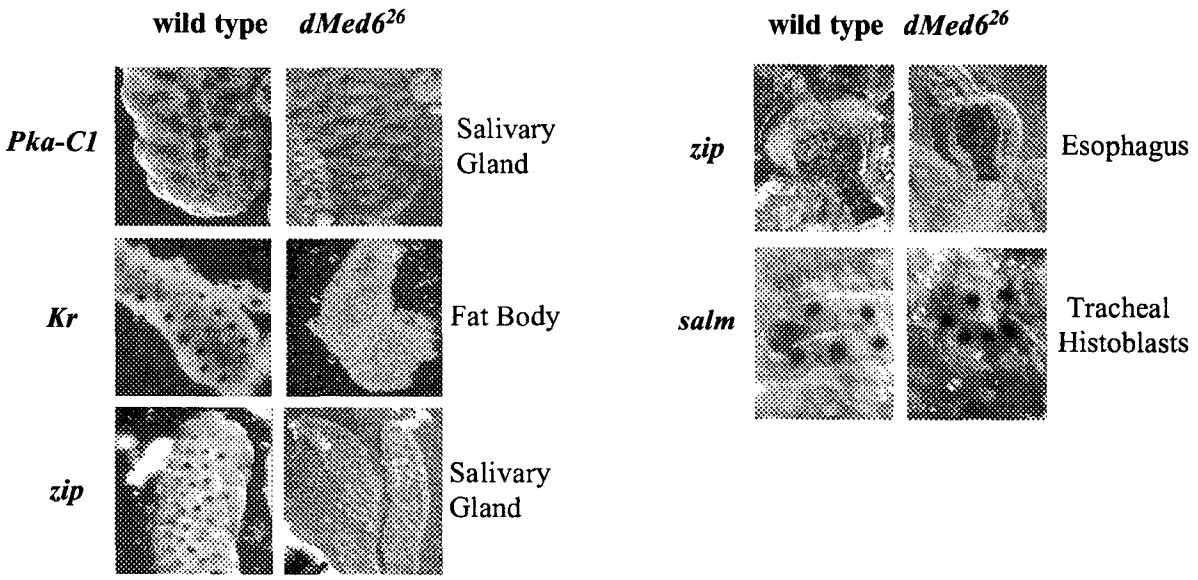


cDNA microarray analysis of *dMed6* mutant larvae

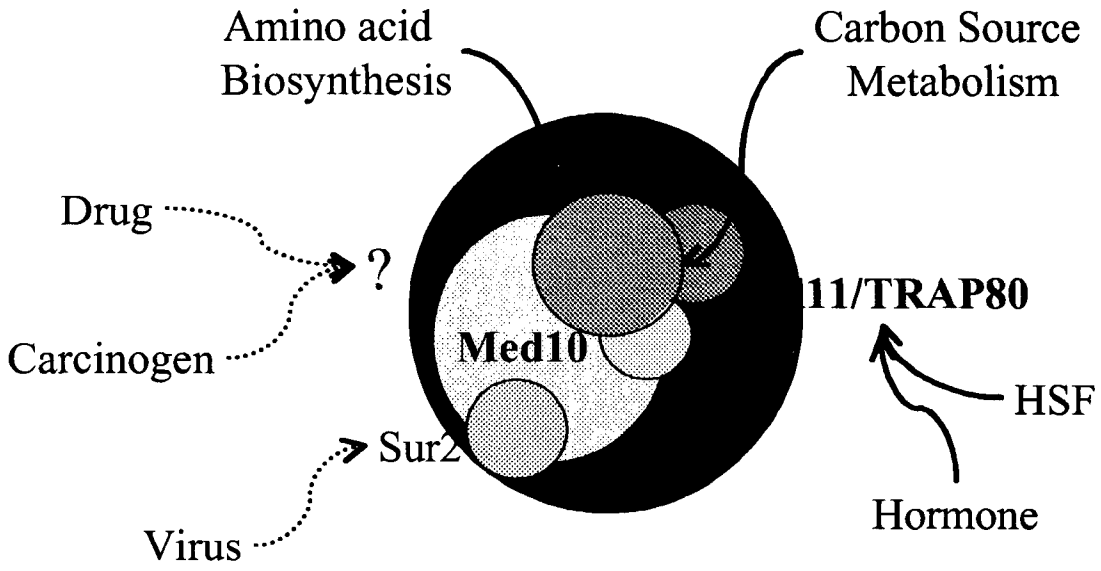
dMed6 is Required for Neuroblast Proliferation



dMed6 is Required for Transcriptional Activation of Distinct Group of Genes In Vivo



Mediator can supply distinct targets for gene-specific control



Acknowledgement