Mediator and its role in non-coding RNA and chromatin regulation

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av

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Avhandlingen baseras på följande delarbeten:

I. **Mediator influences telomeric silencing and cellular life span.**
Zhu X, Liu B, **Carlsten JO**, Beve J, Nyström T, Myers LC, Gustafsson CM.
Mol Cell Biol. 2011 Jun; 31(12): 2413-21.

II. **Mediator promotes CENP-A incorporation at fission yeast centromeres.**
Carlsten JO, Szilagyi Z, Liu B, Lopez MD, Szászi E, Djupedal I, Nyström T, Ekwall K, Gustafsson CM, Zhu X.
Mol Cell Biol. 2012 Oct; 32(19): 4035-43.

III. **Mediator effects on centromere function are not dependent on the exosome subunit Rrp6.**
Carlsten JO, Zhu X, Lopez MD, Gustafsson CM.
Manuscript

IV. **Loss of the Mediator subunit Med20 causes an increase of aberrant RNA polymerase III transcripts in fission yeast.**
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Mediator and its role in non-coding RNA and chromatin regulation

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ABSTRACT

Mediator is a multiprotein complex required for the regulation of RNA Polymerase II (Pol II) transcription. Mediator transmits regulatory signals from activators and repressors to the Pol II machinery at the promoter, but the complex has also many other functions related to control of gene transcription. This thesis aims to expand our knowledge of Mediator's involvement in regulation of the specialized chromatin structures found at telomeres and centromeres as well as its role in regulation of non-coding RNA transcription.

A fine-tuned balance between the histone deacetylase Sir2 and the histone acetyltransferase Sas2 determines the location of the boundary between active and inactive chromatin at budding yeast telomeres. In our work, we demonstrate that Mediator interacts with heterochromatin at telomeres and directs the position of this boundary. Mutations in Mediator subunits cause a depletion of the complex from heterochromatin, which changes the balance between Sir2 and Sas2, and ultimately results in desilencing of subtelomeric regions. Telomeres are important regulators of replicative life span, which is reduced as a consequence of mutations in the Mediator complex.

The *Schizosaccharomcyes pombe* centromeres are also characterized by silent heterochromatin, which is assembled and maintained through a complex multifactorial system. In our work, we find that Mediator is involved in formation of these heterochromatin structures. Loss of the Mediator subunit Med20 causes disruption of heterochromatin and leads to increased transcriptional activity at the centromere. The *med20*Δ mutant also causes reduced levels of CENP-A^{Cnp1}, a centromere specific form of histone H3 found at centromeres, and chromosome instability during cell division. In our work, we find that inactivation of the RNA degrading complex the exosome can reverse the increased levels of pericentromeric transcription observed in *med20*Δ cells, but that it fails to alleviate the chromosome segregation defects. Furthermore, loss of Med20 leads to a changed pattern of siRNA products, which is not further affected in the *med20*Δ*/rrp6*Δ strain. Our results therefore suggest that Mediator and the exosome act in partially independent pathways to influence centromere function.

We also demonstrate that Mediator influences RNA polymerase III (Pol III) transcription. Deletion of *med20*$^{+}$ results in increased transcription of ribosomal protein genes, but also affects Pol III transcription causing an accumulation of aberrant tRNA transcripts with evidence of incorrect transcription termination. The aberrant transcripts are polyadenylated and targeted for degradation by the exosome. The effects of Mediator on Pol III transcription are distinct from those involving Maf1, the classical repressor of Pol III activity. Based on our findings we suggest that fission yeast Mediator takes part in a pathway that coordinates expression of ribosomal protein genes with Pol III transcription.

Work in this thesis demonstrates that Mediator regulates the chromatin structure of several regions characterized by silenced chromatin. Mediator mutations cause loss of heterochromatin at both telomeres and centromeres, which has implications for replicative aging and cell division. Our observation of chromosome segregation defects in *med20*Δ cells may also have more general implications. Chromosomal instability is a driving force in tumorigenesis and mutations in genes encoding Mediator subunits have been linked to the development of several forms of cancer. The thesis also introduces the unexpected finding that Mediator influences Pol III transcription. All together, our results support the view that Mediator does not only mediate signals from gene specific transcription factors to the Pol II transcription machinery. Instead Mediator is a multifaceted protein complex involved in many processes connected to transcription.

Keywords: Mediator, transcription, heterochromatin, centromere, telomere, tRNA, exosome