The THO complex component Tho2 participates in the yeast nuclear mRNP quality control through recruitment of Rrp6

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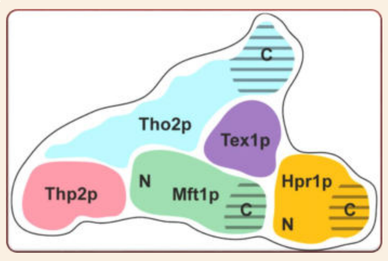
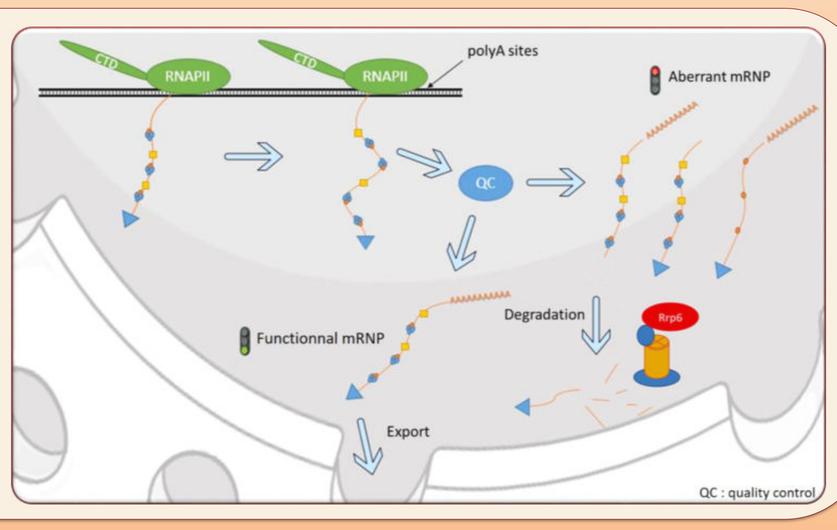
Introduction

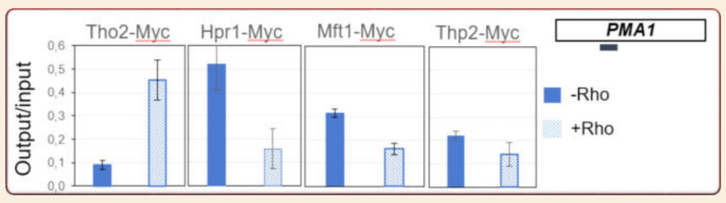


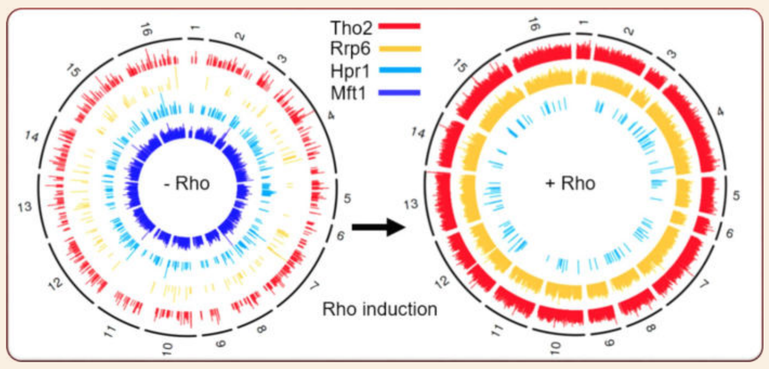
THO complex subunits and global structure. Tex1 is considered as a fifth subunit although it is not required for the complex stability. The THO complex dimerize in vivo (*Schuller et al. 2020).* Taken from *Poulsen, Sanderson et al. 2014.*

In eukaryotes, nascent mRNAs are coated with various proteins and are processed into an export-competent messenger ribonucleoprotein particle (mRNP). Aberrant mRNPs that fail to pass the quality control steps are retained in the nucleus and eliminated by the 3'-5' exonuclease activity of the exosome subunit Rrp6. A key component for mRNP formation is the THO complex, a conserved eukaryotic complex important for transcription elongation and RNA processing, the disruption of which leads to an accumulation of aberrant mRNPs in the nucleus. For the past decade, our team used the bacterial helicase Rho to disrupt mRNP biogenesis in yeast. The disruption of the THO complex leads to the same effects observed upon the induction of Rho in yeast. As Rho has the ability to remove the RNA interacting protein from nascent transcripts, we hypothesized that Rho impacted THO complex recruitment to the chromatin upon its induction. In this work, we studied the genome-wide recruitment of the THO complex subunits during global perturbation of mRNP biogenesis induced by expression of Rho helicase in yeast cells. We monitored the recruitment of Rrp6 as a tool to link the THO complex to aberrant mRNP targeting by the exosome.



Perturbation of mRNP biogenesis leads to a redistribution of THO complex components



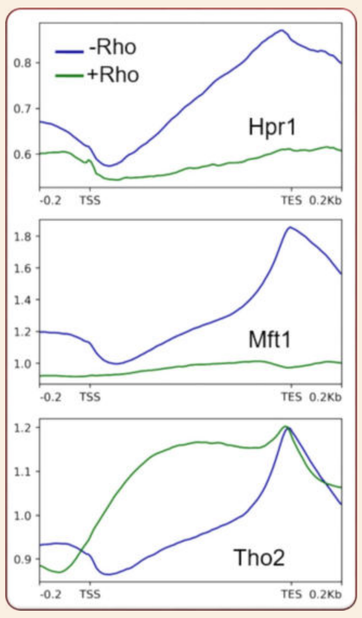


← The recruitment of all THO complex subunits on the PMA1 gene locus is reduced upon Rho induction except for Tho2. This phenomenon was already observed previously by our team for Mft1. *(Stuparevic, Mosrin-Huaman et al. 2013)*

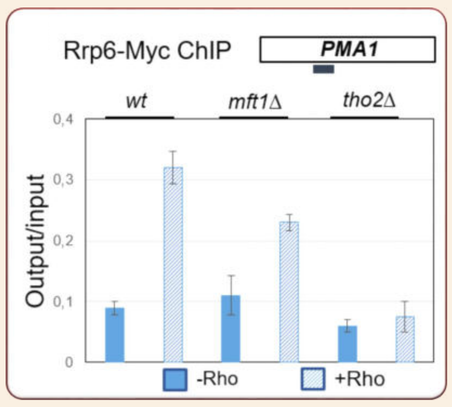
→ The metaprofiles of chromatin recruitment drawn from ChIP-seq datasets of Hpr1, Tho2 and Mft1 subunits along 5752 mRNA coding loci are typical of the THO complex in physiological condition *(Meinel, Burkert-Kautzsch et al. 2013)* but are lost upon Rho induction, except for Tho2. Tho2 is highly recruited from the start site of the transcription (TSS) until the end site (TES), in opposition to its typical profile.

← The THO subunits Hpr1 and Mft1 but not Tho2 are evicted from the chromatin upon Rho induction on the whole genome. Tho2 is more recruited on the chromatin upon Rho induction. Rrp6 is also more recruited, as previously documented by our team. *(Moreau, Le Dantec et al. 2019)*

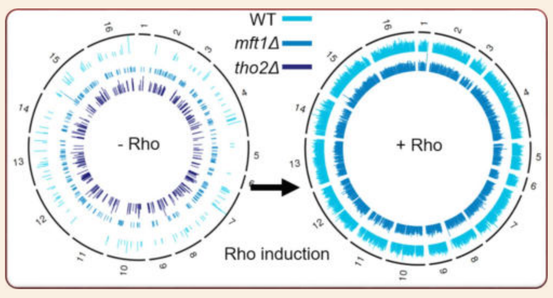
→ Upon Rho induction, the THO complex dissociates from the chromatin but Tho2 is independently recruited back



The removal of Rho-induced aberrant mRNPs requires Tho2

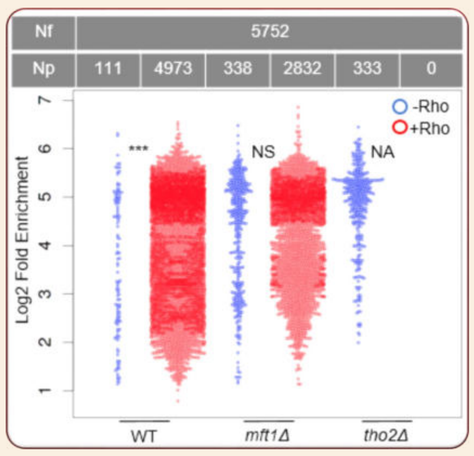


← The recruitment of Rrp6 on the PMA1 loci is Tho2 dependent. The induction of Rho in WT and mft1Δ cells leads to the increased recruitment of Rrp6. In tho2Δ cells, this induced-recruitment is not visible.



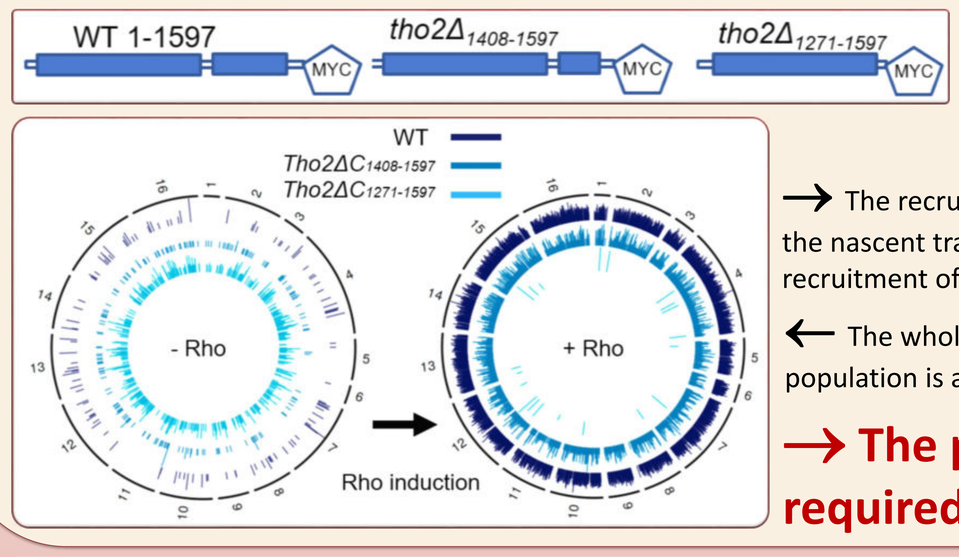
→ Beeswarm showing the interaction of Rrp6 with the chromatin in WT, mft1Δ and tho2Δ cells. Upon Rho induction, Rrp6 is highly recruited on the 5752 mRNA coding loci in both WT and mft1Δ cells , but is not in tho2Δ cells

← The loss of the Rho–induced Rrp6 recruitment in tho2Δ cells is generalized on all 16 yeast chromosomes, reflecting a global impact of the removal of Tho2 on the aberrant mRNP degradation process.



→ The depletion of Tho2 leads to the absence of recruitment of Rrp6 upon mRNP biogenesis perturbation by Rho

Tho2 C-terminal domain is required to recruit Rrp6

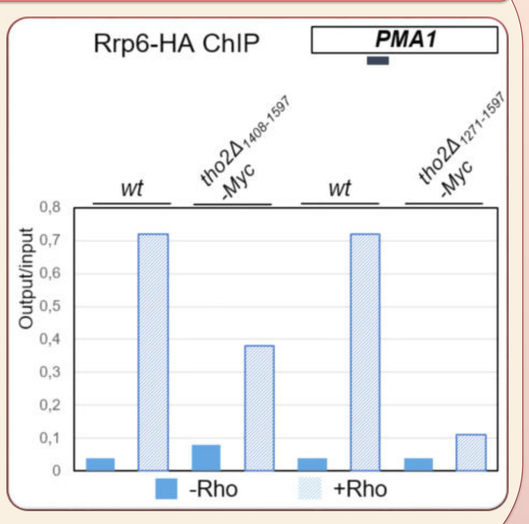


← Tho2 C-terminus harbors a RNA-interacting domain which allow the interaction of THO with the nascent mRNA. Two Tho2 mutants were generated for this study. *tho2Δ*$_{1408\text{-}1597}$ has a weakened RNA-interaction ability while *tho2Δ*$_{1271\text{-}1597}$ has none.

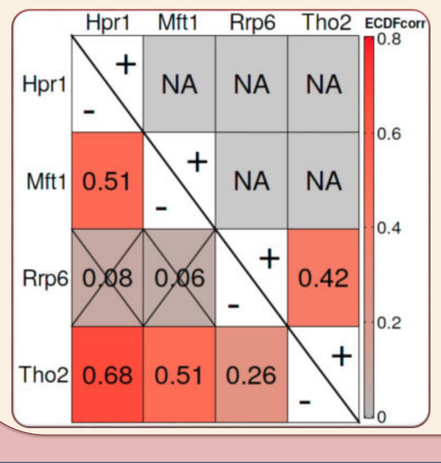
→ The recruitment of Rrp6 on the PMA1 loci depends on the ability of Tho2 to interact with the nascent transcript. If the partial deletion of the C-terminal domain of Tho2 hinders the recruitment of Rrp6 upon Rho induction, its total removal completely inhibits this recruitment.

← The whole Rho-induced Rrp6 recruitment on the entire yeast mRNA coding loci population is affected by the loss of the RNA-interaction function of Tho2.

→ The presence of Tho2 on the aberrant transcripts is required for their targeting and degradation by Rrp6



Hints of Rrp6 and Tho2 cooperation upon Rho induction



← We measured the distance between Rrp6 and THO subunits chromatin interaction sites in both « –Rho » and « +Rho » conditions. We obtain correlation factors indicating their overall proximity. In « -Rho » condition, all THO subunits show a strong proximity to each other [0,51 - 0,68], while having a low proximity factor with Rrp6. Upon Rho induction, THO subunit cohesion is lost but Tho2 shows a high proximity with Rrp6 (0,42).

→ Functional cooperation between Tho2 and Rrp6, maybe physical ?

Conclusion/Model