Investigating the role of INO80 chromatin remodeller in the metabolic stress response of *S.cerevisiae*

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Aims

- To illuminate the role of the INO80 chromatin remodelling enzyme in regulation of the transcriptional response to metabolic stress
- To identify how INO80 controls the expression of General Amino-Acid Control (GAAC) genes under metabolic stress conditions.

Introduction

ATP-dependent chromatin remodelling enzymes unfold and shape the chromatin landscape and are involved in all major chromatin based processes. While their role in regulating initiation of transcription is well established, their involvement in coordination of the transcriptional response to metabolic stress is poorly understood[(1)].

In this project, we set out to understand the role of the ATP-dependent chromatin remodelling enzyme INO80 which has been suggested to work in various metabolic pathways, under metabolic stress conditions. In response to nitrogen starvation, the INO80 complex localises at the body of GCN4-regulated amino acid biosynthesis genes (GAAC). GAAC genes are strongly induced in response to metabolic stress however the role of INO80 at these genes is unclear since RNA levels are largely unchanged in the absence of INO80[(2)].

Under metabolic stress conditions, the high levels of RNA don't appear to be translated as protein levels continue to decrease over time. When these conditions are removed, the mutant cells never recover suggesting a role for INO80 in the recovery of protein levels post-starvation.

Currently, INO80 is known to regulate RNA quality control[(2)] therefore we hypothesised that INO80's role in the metabolic stress response may be to produce stable (translatable) mRNAs.

Acknowledgements

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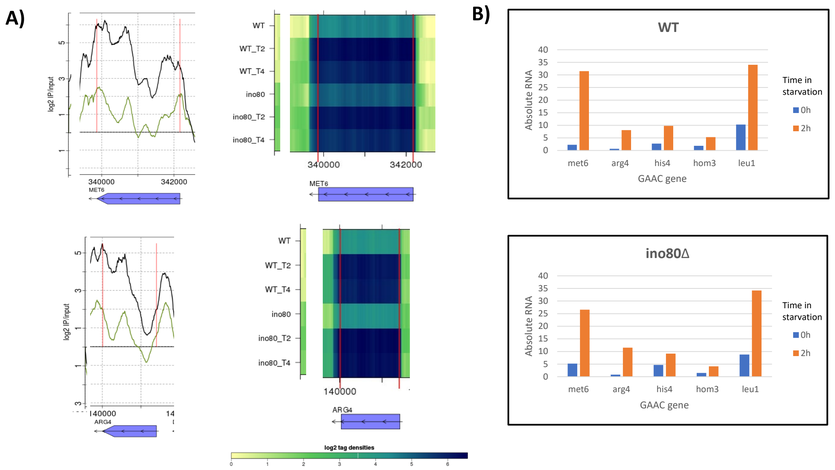
References

1. Shen X, Mizuguchi G, Hamiche A, Wu C. (2000). A chromatin remodeling complex involved in transcription and DNA processing. Nature. 406. 541-4. 10.1038/35020123.
2. Poli J, Gasser SM, Papamichos-Chronakis M. 2017. The INO80 remodeller in transcription, replication and repair. Phil. Trans. R. Soc. B 372: 20160290. http://dx.doi.org/10.1098/rstb.2016.0290

Results

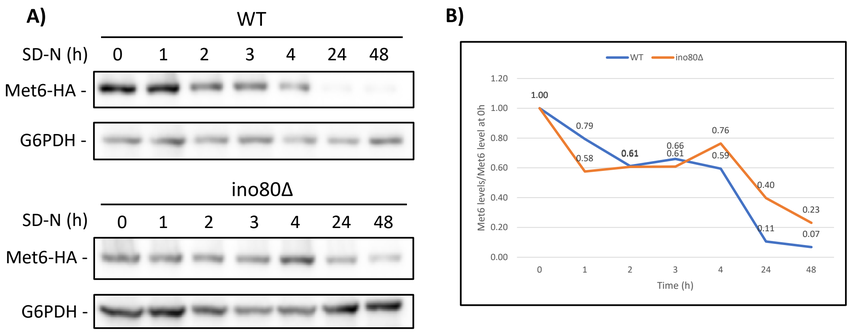
1.Upon nitrogen starvation, INO80 is recruited at GAAC genes but is not involved in their transcriptional activation.



a) ChIP-seq (left) and RNA-seq (right) data. ChIP-seq shows the binding of INO80 to the MET6 and ARG4 genes. RNA-seq shows the large increase in RNA levels in both strains indicated by the deep blue colour on the heat map.
b) Absolute RNA levels based on RNA-seq data, before and after nitrogen starvation (SD-N) in both wild type (top) and ino80Δ strains. All show an increase in RNA levels after 2h.

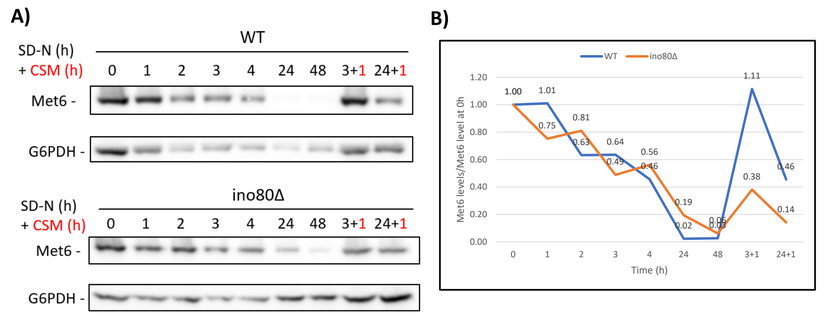
* All data presented in figure 1 was not produced by me and is used with the permission of the Manolis Papamichos lab (Shapira et al, *unpublished*)

2. Long-term decrease of protein levels over time in starvation conditions is partially dependent on INO80.



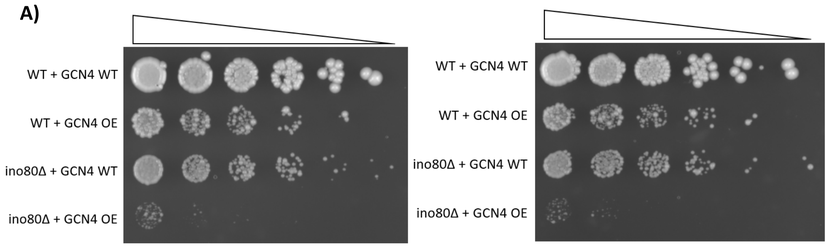
a) Western blots showing the levels of HA-tagged Met6 protein over a time-course under nitrogen-starvation conditions (SD-N medium). G6PDH was used as a loading control.
b) Quantification of MET6-HA protein levels following nitrogen starvation based on western blot band intensities. Data was normalised with loading control G6PDH. The graph indicates reduction in protein levels, with the largest decrease occurring within the first 2 hours of incubation in SD-N medium.

3. INO80 is required for the recovery of GAAC protein levels following return to rich-media.



a) Western blots showing a second replicate of the initial time-course with HA-tagged Met6 but with 2 extra samples. These samples (3+1 and 24+1) were removed from SD-N at the indicated time and put back into nutrient-rich media (CSM) for 1 hour to determine whether or not protein levels of Met6 would recover.
b) Quantification of Met6-HA protein levels following nitrogen starvation and subsequent nutrient-rich media (CSM) based on western blot band intensities. Data was normalised with loading control G6PDH. The data reinforces the initial findings of protein decline over time in both strains. However, after resuspension in CSM the WT cells recover and protein levels increase dramatically whereas protein levels in the *ino80Δ* cells are not.

4. INO80 becomes essential for viability in cells over-expressing the GAAC activator, GCN4.



a) Serial dilution spotting plates of WT and *ino80Δ* strains with both wild-type GCN4 (GCN4 WT) and overexpressed GCN4 (GCN4 OE). The 2 replicates are shown above and clearly indicate overexpression of GCN4 affects the ability of the WT to grow. However, the combination of ino80Δ and GCN4 overexpression appears to be lethal with very little growth.

Conclusions

- The INO80 complex does not affect the constitutive levels of GAAC proteins but appears to promote their dynamic turnover and synthesis.
- INO80 is required for cell growth and viability in cells with ectopic upregulated GAAC gene expression.
- Further studies will be necessary to understand the exact INO80 mechanism in response to metabolic stress.