Some Aspects of Chromatin Remodeling in Yeast

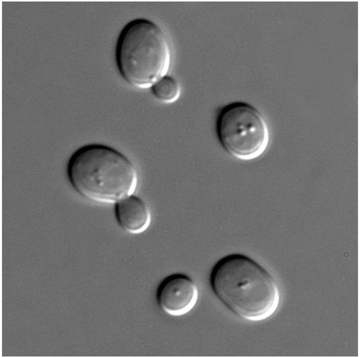
collaborative work with Christoph Schueller and Eva Klopf (BOKU, Tulln)

Heiko A. Schmidt

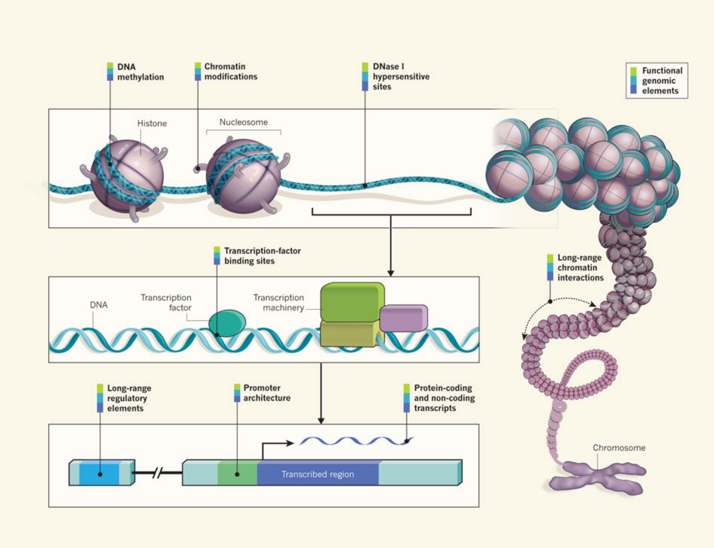
Center for Integrative Bioinformatics Vienna (CIBIV)
Max F. Perutz Laboratories (MFPL), Vienna, Austria

Object of study

- Saccharomyces cerevisiae (yeast)
- Genome size: about 12 Mb
- Number of genes: about 7000
- Genome resource: Saccharomyces Genome Database (SGD) `yeastgenome.org`
- . . . and we are interested in aspects of nucleosome placement by the INO80 chromatin remodeling complex.

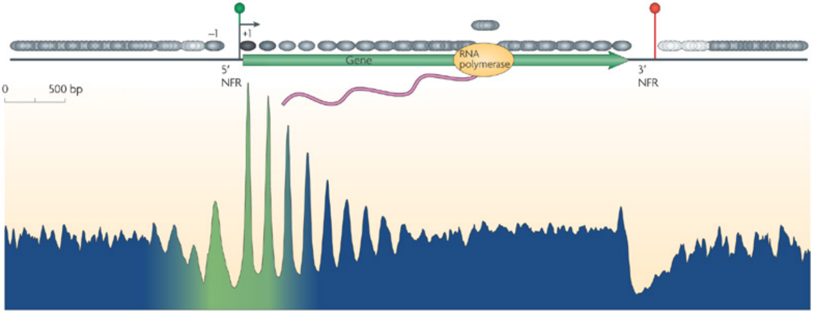


Metagenomic Regulation



Gene expression is influenced by various factors: chromatin state, histone modifications, DNA methylation, but also nucleosome placement.

Nucleosome Positioning in Regulation



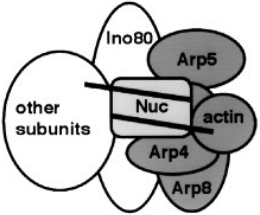
Nature Reviews | Genetics

There is high correlation of nucleosome placement at the transcription start site (TSS).

- the 5'-NFR is the place where the polymerase binds
- the $+1$ nucleosome position covers the TSS
- during transcription position $+1$ is depleted
- after a short time a nucleosome is replaced at $+1$ blocking/reducing further transcription

INO80 complex / ARP8^{-}

- The INO80 complex is involved in nucleosome placement
- ARP8 is part of the INO80 complex
- current hypothesis: INO80 is recruited by the polymerase and plays a role in remodeling chromatin after passing
- expected effect of ARP8^{-}: INO80 function is hampered or slowed down



Experimental design

- two cell lines: WT, ARP8^{-}

Experimental design

- two cell lines: WT, ARP8$^-$
- stress induction by salt
  (only as a well-known means to induce genes)

Experimental design

- two cell lines: WT, ARP8^{-}
- stress induction by salt
  (only as a well-known means to induce genes)
- samples taken at 0’ (control), 10’, 20’, 30’, 45’, 60’

Experimental design

- two cell lines: WT, ARP8$^-$
- stress induction by salt
  (only as a well-known means to induce genes)
- samples taken at 0' (control), 10', 20', 30', 45', 60'
- 50' MNase digest before hybridization

Experimental design

- two cell lines: WT, ARP8^{-}
- stress induction by salt
  (only as a well-known means to induce genes)
- samples taken at 0' (control), 10', 20', 30', 45', 60'
- 50' MNase digest before hybridization
- NuSA-chip: nucleosomes are collected and the bound DNA snippets extracted
- (single color) hybridization to high-density tiling array

Experimental design

- two cell lines: WT, ARP8^{-}
- stress induction by salt
  (only as a well-known means to induce genes)
- samples taken at 0' (control), 10', 20', 30', 45', 60'
- 50' MNase digest before hybridization
- NuSA-chip: nucleosomes are collected and the bound DNA snippets extracted
- (single color) hybridization to high-density tiling array
- in addition mRNA was extracted and also hybridized to the high-density tiling array

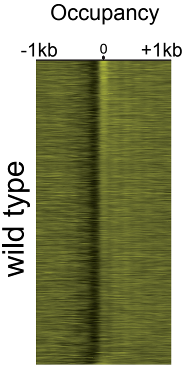
Data

- High density tiling arrays:
    - sequence length of tiles: 25bp
    - step size: 8bp
    - average overlap: 25 - 8 = 17bp
- normalized log2 intensity data for each tile (if not cross-hybridizing) for each of the sub-experiments

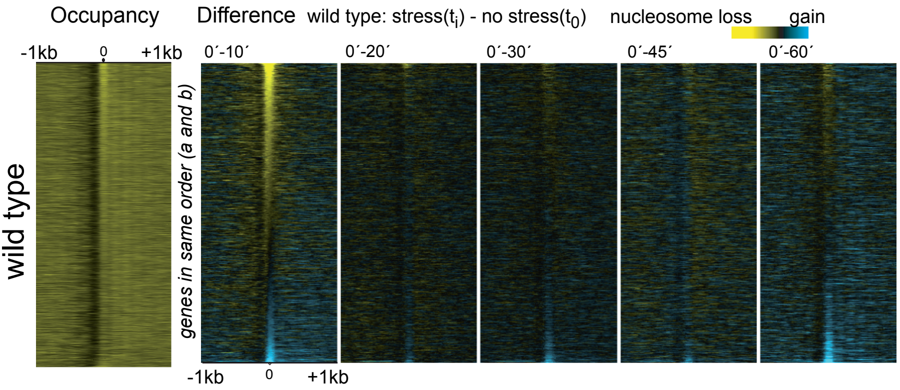
Data

- High density tiling arrays:
  - sequence length of tiles: 25bp
  - step size: 8bp
  - average overlap: 25 - 8 = 17bp
- normalized log2 intensity data for each tile (if not cross-hybridizing) for each of the sub-experiments
- we collected the values +1000/-1000bp around the +1 nucleosome

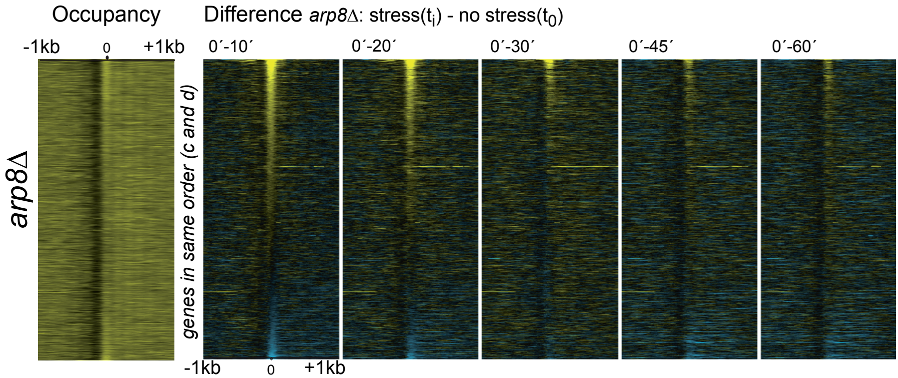
Nucleosome levels in WT



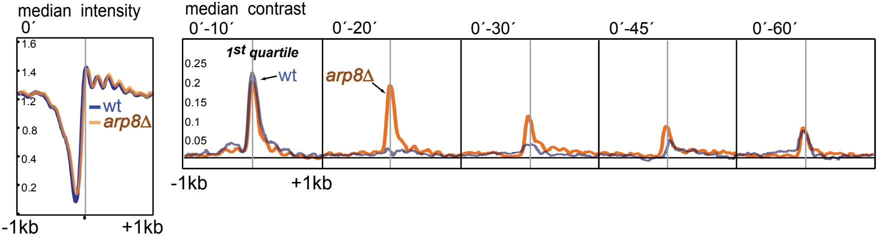
Nucleosome levels in WT



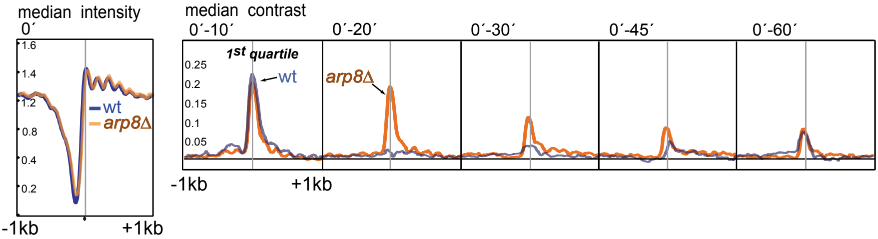
Nucleosome levels in ARP$^-$



Summary of the changes in WT and ARP^{-}

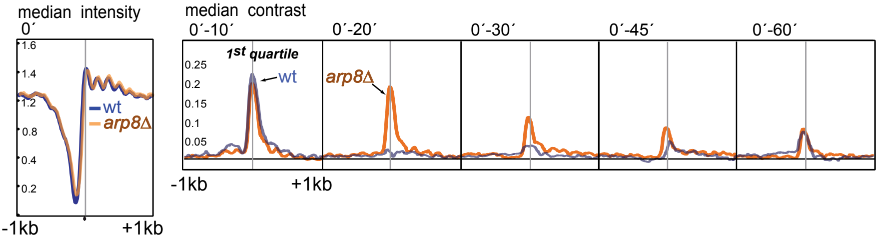


Summary of the changes in WT and ARP$^-$



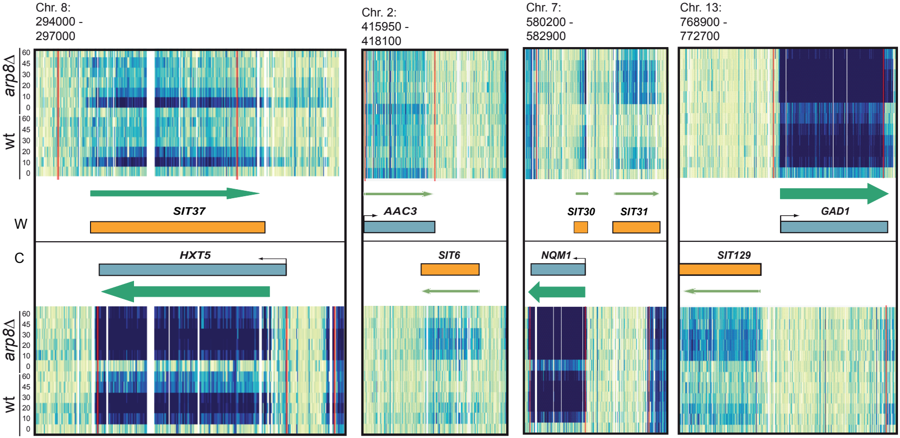
- Conclusion: INO80 acts mainly on the re-placement of the +1 nucleosome.

Summary of the changes in WT and ARP⁻



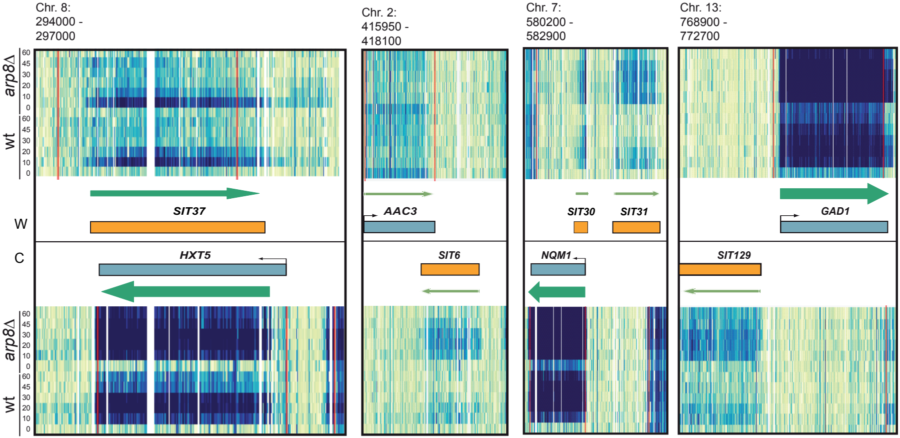
- Conclusion: INO80 acts mainly on the re-placement of the +1 nucleosome.
- Effect: the expression levels stay high longer in the ARP8⁻.

(Cryptic) Stress-Induced Transcripts (SITs)



- In addition, using the mRNA data, we were able to find a number of unknown/non-annotated transcripts

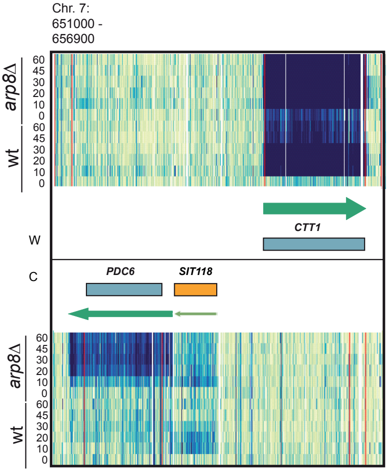
(Cryptic) Stress-Induced Transcripts (SITs)



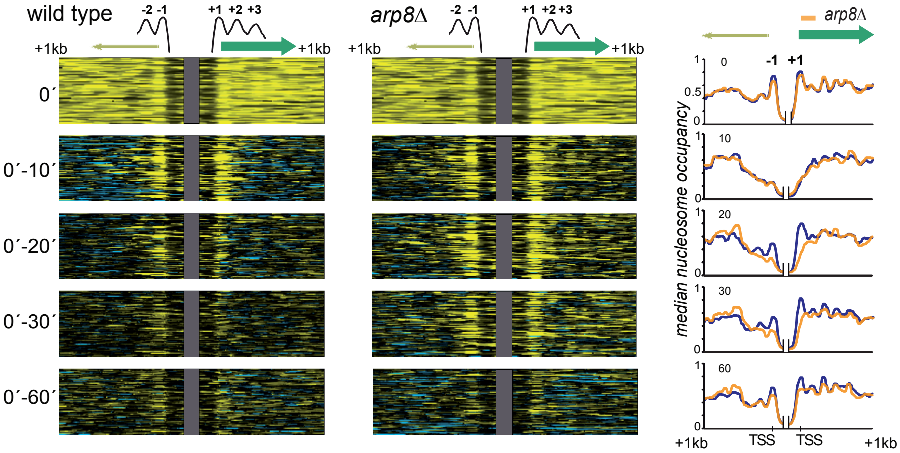
- In addition, using the mRNA data, we were able to find a number of unknown/non-annotated transcripts
- which typically do not even contain any ORF, thus, not being coding.

Bi-directional promotors

- many of these SITs upstream of strongly stress-induced genes
- placed in opposite direction, thus, sharing the same promotor

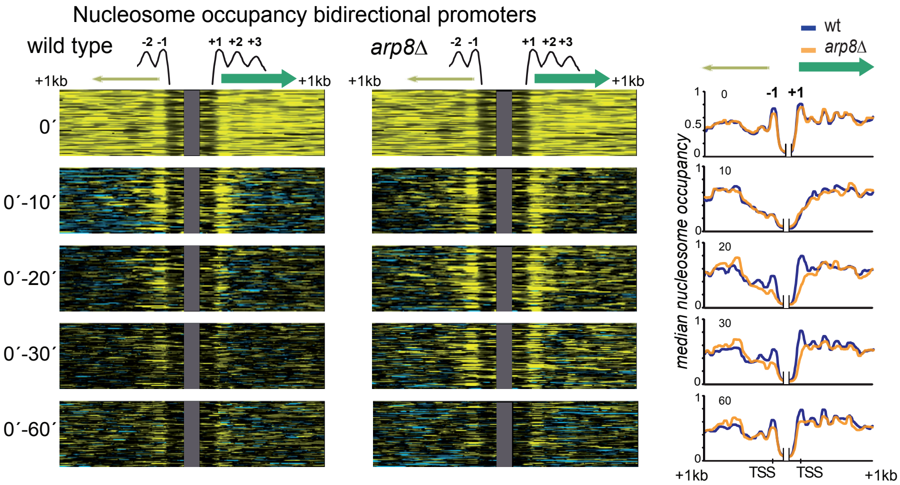


Nucleosome positioning around bi-directional promotors



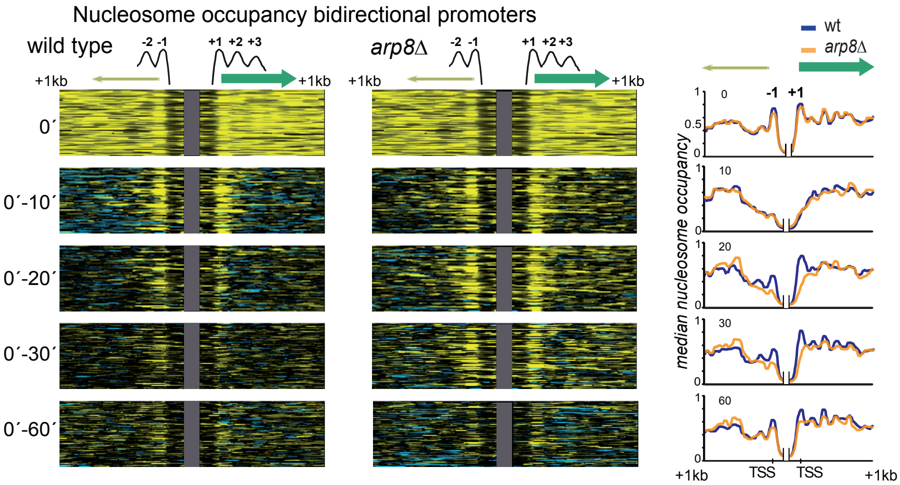
Nucleosome occupancy bidirectional promoters

Nucleosome positioning around bi-directional promotors



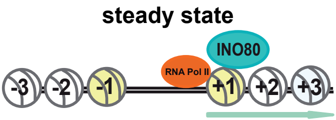
- Nucleosome structure is less pronounced towards the weaker transcript

Nucleosome positioning around bi-directional promotors



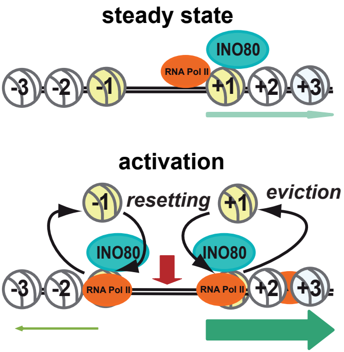
- Nucleosome structure is less pronounced towards the weaker transcript
- Other than that both sides behave the same as other induced transcripts/genes.

Conclusions



- INO80 is recruited by RNA Pol II during transcription

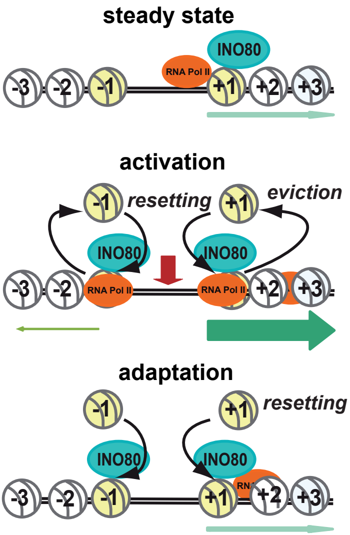
Conclusions



- INO80 is recruited by RNA Pol II during transcription

- INO80 is involved in re-instating the +1 nucleosome which is repeatedly evicted by RNA Pol II
- (the same in one- or bi-directional promotors)

Conclusions



- INO80 is recruited by RNA Pol II during transcription

- INO80 is involved in re-instating the $+1$ nucleosome which is repeatedly evicted by RNA Pol II
- (the same in one- or bi-directional promotors)

- as the cell adapts re-instating the $+1$ nucleosome supporting down-regulating transcription
- Hence, transcription stays on longer in the ARP8^{-} mutant (because the $+1$ nucleosome is re-instated later).