universität wien

# DIPLOMARBEIT

Titel der Diplomarbeit

## The Chromatin Remodeling Complex Ino80-C and its Role in Stress Gene Transcription

angestrebter akademischer Grad

Magister/Magistra der Naturwissenschaften (Mag. rer.nat.)


| | |
|---|---|
| Verfasserin / Verfasser: | Eva Klopf |
| Matrikel-Nummer: | A9901335 |
| Studienrichtung (lt. Studienblatt): | Molekulare Biologie |
| Betreuerin / Betreuer: | Dr. Christoph Schüller |


Wien, am 2.12. 2007

Danksagung

Ich danke Ludmila Paškova, Andreas Roetzer, Christoph Schüller, Andriy Petryshin, Jiři Veis, Veerle de Wever, Nikola Winter und allen anderen Mitgliedern der Kragler-Gruppe, Harald Nierlich, Erna Huber, Christian Bernhart und dem gesamten Department für Biochemie am MFPL.

Mein besonderer Dank gilt meiner Familie, insbesondere meinen Eltern Berndt und Gudrun, die mich meine gesamte Studienzeit hindurch unterstützt haben und meinem Partner Thomas, der immer für mich da war.

# Contents

# Zusammenfassung

Ino80-C ist ein Chromatin remodeling Komplex, der sowohl bei der Reparatur von Doppelstrangbrüchen als auch in Prozessen der transkriptionellen Kontrolle verschiedener Gene eine wichtige Rolle spielt. Sind bestimmte Untereinheiten dieses Komplexes defekt führt dies zu erhöhter basaler mRNA Konzentration diverser Stressgene und auch zu deren verlängerter Expression. Fehlt beispielsweise die Ino80-C Untereinheit Arp8, ist eine Vielzahl von Stressgenen signifikant überexprimiert. Arp4 und Ino80 sind ebenfalls Untereinheiten von Ino80-C. Nur während der aktiven Transkription können diese beiden Proteine in Promoter Regionen und offenen Leserahmen von Stressgenen detektiert werden. Ist die RNA Polymerase II durch ein temperatursensitives Allel der Untereinheit Rpb1 (rpb1-1) deaktiviert findet keine Assoziation von Ino80 und Arp4 mehr statt. Folglich ist Elongation ein Signal für die Rekrutierung des Ino80 Komplexes.

In dieser Arbeit wurde auch die Funktion von Ino80-C während der aktiven Transkription von Stressgenen untersucht. Während der Elongation ist die Dissoziationkinetik von Nukleosomen im Wild Typ und bei Mutanten von Ino80-C gleich. Allerdings ist die Reassoziation der Histone H3 und H4 am offenen Leserahmen des Stressgens STL1 signifikant verlangsamt.

Ino80-C wird beim Auftreten von Doppelstrangbrüchen durch die Phosphorylierung von Histon H2A (γH2A) rekrutiert. In dieser Arbeit konnte festgestellt werden, dass Ino80-C während der Transkription nicht durch γH2A zu den jeweiligen Loci rekrutiert wird.

Die Funktion von Ino80-C ist unabhängig von der Art des Stresses (osmotischer Stress, Hitzestress, Kupferionen) und den beteiligten Aktivatoren. Deshalb ist es sehr wahrscheinlich, dass Ino80-C an der Repression stark exprimierter Gene beteiligt ist. Mutanten des Histon Chaperones Asf1 und der Histon Methyltransferase Set2 weisen einen ähnlichen Phänotyp auf wie Ino80-C Mutanten. Demnach könnten diese drei Faktoren am selben Prozess beteiligt sein.

# Abstract

The yeast chromatin remodeling complex Ino80-C is involved in transcription and DNA double strand break (DSB) repair. We report here an Ino80-C function required for proper regulation of stress induced genes. Mutants in Ino80-C subunits have increased basal and sustained induced mRNA levels of several stress genes. Importantly, transcripts are globally increased in an *arp8*Δ mutant after osmotic stress. Concomitant with induction of transcription, Ino80 and Arp4 are recruited to the promoter and open reading frame regions of stress genes. Active elongation by RNA Polymerase II (RNA Pol II) is required for association of Arp4 and Ino80 with the ORF regions. A conditional inactive allele of the largest Pol II subunit Rpb1 (*rpb1-1*) prevented Arp4 recruitment at the restrictive temperature. Furthermore, cells lacking functional Arp4 have a greatly diminished reappearance of histone H3 and H4 in the open reading frame of the *STL1* gene during stress induced transcription. However, stress induced nucleosome eviction is not affected. Recruitement of INo80-C to stress genes is not dependent on a mechanism involving γ-H2A as for double strand break recruitment. Ino80-C function is required for down-regulation of stress induced transcripts after different treatments such as high osmolarity (*CTT1, STL1*), heat shock (*HSP82, HSP12*) and copper ions (*CUP1*), all involving different transcriptional activators. Mutants of histone chaperone Asf1 and histone methyltransferase Set2 display a similar phenotype as mutants of Ino80-C. Thus the three factors could possibly work in the same process. Ino80-C has a role in the repression of highly transcribed genes, most probably by re-installing a repressive chromatin structure.

# 1. Introduction

All cells of an organism carry the same genetic information. The interpretation of this information by regulated transcription leads to the establishment of different cell types and phenotypes such as stress resistance. One major mechanism is the regulated initiation of transcription of RNA Polymerase II. All DNA of the nucleus is complexed with Histone octameres forming chromatin a structure opposing transcription by Pol II. The transcription cycle of RNA polymerase II (RNA Pol II) has four stages defined by the phosphorylation state of its C- terminal domain (CTD): initiation, promoter escape, elongation and termination. RNA Pol II and its associated basal transcriptional machinery is assisted by a variety of factors that make chromatin more accessible: Several enzymes are able to modify N-termini of histones covalently (e.g. histone acetyltransferases or methylases) to facilitate mobilization of nucleosomes or support binding of other factors. ATP- dependent chromatin remodeling factors are able to alter the nucleosome position and thereby enable exposition of DNA to transcription (Workman and Kingston, 1998; Narlikar et al., 2002).

## 1.1. Transcription Initiation through the Nucleosome Barrier:

Initiation of transcription starts with the binding of activators upstream of the promoter leading to recruitment of coactivator complexes which facilitate stronger binding of activators. These coactivators are chromatin remodeling complexes (Swi/Snf), histone- modification enzymes (Gcn5, Esa1) and the mediator complex making nucleosomal DNA elements more accessible to general transcription factors. Moreover the histone- variant Htz1 flanks a 200bp nucleosome free region at the silent promoter which marks the site for RNA Pol II pre-inititation complex (PIC) formation (Figure I1) (Kobor et al., 2004; Krogan et al., 2003a; Mizuguchi et al., 2004; Zhang et al., 2005a). After positioning of the preinitiation complex, limited melting of the DNA duplex (11-15bp) takes place resulting in a single stranded template. The C- terminal domain (CTD) of the polymerase is phosphorylated on two serine residues which leading to loss of association to the General Transcription Factors before entering elongation. Depending on the phosphorylation status of Serine2 and Serine5 the CTD is also a domain recruiting several factors enabling Pol II elongation.

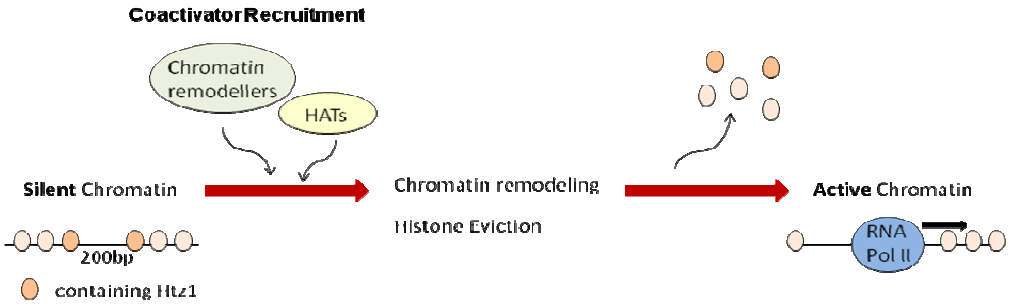



**Figure I1** Activity of chromatin remodellers and histone acetyl transferases lead to chromatin remodeling and histone eviction which enables switch from silent to active chromatin (Adapted from Li et al. Cell 128, 707–719, February 23, 2007 ª2007 Elsevier Inc.) For more details see Appendix Figure A1.

## 1.2 Transcription Elongation through the Nucleosome Barrier

During elongation RNA Polymerase II recruits several factors facilitating passage through the nucleosome barrier. Among them are chromatin modifyers, chromatin remodeling complexes and histone chaperones, all three groups having important and distinct functions.

The most prominent modifications on chromatin during elongation are acetylation of Histone 3 and 4, methylation of H3 at K4, K36 and K79 (Krogan et al., 2003a; Krogan et al., 2003b; Ng et al., 2003) and ubiquitination of histone H2B K123 and K120 (see Table1). Histone H3 trimethylation on lysine 36 (K36) mediated by Set2 is an important landmark during elongation (Li et al., 2003) and is globally correlated with transcription (Pokholok et al., 2005).

The ATP- dependent chromatin remodeling complexes Swi/Snf and RSC, Mi-2(CHD), ISWI and Ino80-C have a function for helping Pol II pass through nucleosomes (Mellor, 2006; Workman and Kingston, 1998). Analysis of their contribution to global transcription suggests a largely distinct role for each of them during elongation (see section 1.5.).

Transcription removes (evicts) nucleosomes from transcribed regions (Schwabish and Struhl, 2004). Thus a third group of factors is recruited by elongating RNA Pol II, the so- called histone chaperones. Histone chaperones support the rapid eviction of histones in front of elongating RNA Pol II (Belotserkovskaya et al., 2003; Mason and Struhl, 2003; Orphanides et al., 1998; Orphanides et al., 1999; Reinberg and Sims, 2006) but are also involved in reassembly. Asf1 was shown to evict and deposit H3 and H4 whereas FACT, a dimer of Spt16 and Pob3/SSRP1 facilitates disassembly and reassembly of H2A/H2B dimers during Pol II transcription. Additionally, Spt6 contributes maintaining normal chromatin structure during elongation (Kaplan et al., 2003).

PAF, a multisubunit complex is one of the first factors associating with the phosphorylated CTD of RNA Pol II (reviewed in Rosonina and Manley, 2005). It is critical for the recruitment

of several chromatin modifyers like the H3K4 methyltransferase Set1 (function will be discussed later) and facilitates H2B ubiquitination by Rad6/ Bre (see Figure I2). The attraction of the histone chaperones Spt6 and FACT is also dependent on the function of PAF. The activity of Set2 is independent of PAF. Set2 directly interacts with Ser2-phosphorylated CTD, thus methylating H3K36 at the 3' end of transcribed genes.

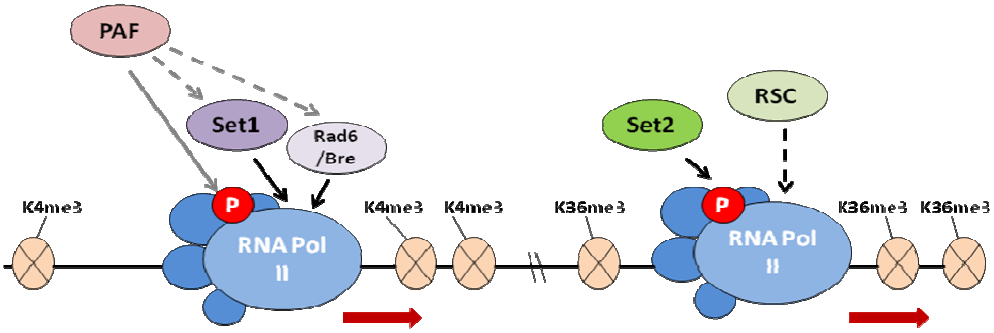



**Figure I2** The chromatin landscape during elongation is determined by the factors associated with different forms of Pol II. PAF facilitates the binding of FACT, COMPASS, and Rad6/Bre1 to the Ser5-phosphorylated CTD, which results in H2B ubiquitination and accumulation of trimethylation of H3K4 at the 50 end of ORF. (Adapted from Li et al. Cell 128, 707–719, February 23, 2007 ª2007 Elsevier Inc.)

## 1.3. Histone Modifications and Transcription

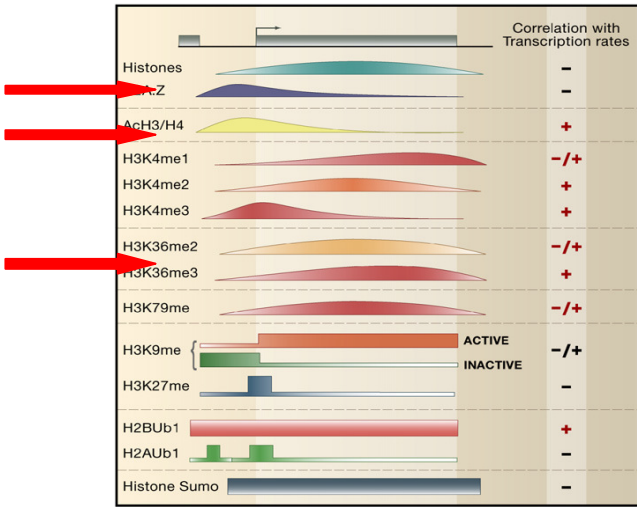



**Figure 3** Distribution of modifications in coding regions and promoters. The histone variant Htz1 (H2A.Z ) is preferentially found in the promoter region as well as acetylated H3 and H4. Trimethylated K36 on H3 is found more in the 3' region of open reading frames (Taken from Li et al. Cell 128, 707–719, February 23, 2007 ª2007 Elsevier Inc.)

Histones undergo a variety of modifications after translation such as acetylation, phosphorylation, sumoylation, ubiquitination, methylation and ADP- ribosylation. Acetylation as well as methylation of H3K4, H3K36 and H3K79 are characteristic for active transcription and thus are euchromatin modifications whereas methylation of H3K9 and H3K27 are more

localized to inactive regions. The localization of these patterns is tightly regulated and also highly conserved because of its dramatic effect on transcription (Figure 3). Chromatin modifyers are the first class of factors described here involved in changing the chromatin structure to make DNA more accessible.

| **Conserved Chromatin Modifications and corresponding Enzymes** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Modification** | **Position** | **Enzymes** | | | | **Recognition Module(s)** | **Functions in Transcription** |
| | | ***S. cerevisiae*** | ***S. pombe*** | ***Drosophila*** | **Mammals** | | |
| Methylation | H3 K4 | Set1 | Set1 | Trx, Ash1 | MLL, ALL-1 Set9/7 ALR-1/2 ALR, Set1 | PHD, Chromo, WD-40 | Activation |
| | H3 K36 | Set2 | Clr4 | | HyPB, Smyd2, NSD1 | Chromo (Eaf3)m, JMJD | Repression |
| | H3 K79 | Dot1 | | | Dot1L | Tudor | Activation |
| Ubiquitination | H2B K120/ K123 | Rad6, Bre1 | Rad6 | | UbcH6; RNF 20/40 | (COMPASS) | Activation |
| Acetylation | H4/K16 | NuA4 | | dMOF | hMOF | Bromodomain | Activation |
| | Htz1/K14 | SAGA, NuA4 | | | | | Activation |

**Table 1** Conserved histone modifications in *S. cerevisiae*, *S. pombe*, *Drosophila melanogaster* and Mammals**.** (Adapted from Li et al. Cell 128, 707–719, 2007.) For more details see Appendix Table A1

### 1.3.1. Acetylation:

The best studied histone modification is acetylation of certain lysin residues. Acetylation is thought to affect chromatin structure by changing the charge of the histone tails (positively charged lysins) and thereby neutralizing the interaction with the (negatively charged) DNA. Furthermore acetylation is a modification recognizable by several other (“secondary”) factors. In general histone acetyltransferases (HATs) like Esa1 (a subunit of NuA4) and Gcn5 (a subunit of SAGA) acetylate lysine residues. Acetylation is a modification connected to active transcription. Histone 3 and 4 are predominantly in an acetylated state when promoters are active. Additionally the level of acetylation is proportional to the level of transcription (Pokholok et al., 2005). However Lindstrom et al. (2006) showed that the activity of the HAT NuA4 in combination with other factors can also have repressive effects on transcription.

1.3.1.1. NuA4:

Yeast NuA4 complex contains 13 associated proteins (reviewed in Carozza et al., 2003; Utley et al., 2003). Esa1, the essential, catalytical subunit of NuA4 (Allard et al., 1999) globally acetylates H4 and H2A. Esa1 and the subunit Arp4, a protein also present in the chromatin remodeling complexes Swr-C and Ino80-C are also involved in repair of DNA double-strand breaks (DSBs) in vivo (Bird et al., 2002). Eaf3 is a subunit also present in the Histone- deacetylase Rpd3S. Absence of Eaf3 leads to globally changed acetylation patterns (Reid et al., 2004). Over coding regions acetylation is dramatically increased (hyperacetylation) whereas the promoter regions are hypoacetylated.
NuA4 has been reported to be mainly involved in active transcription of genes (Galarneau et al.,2000) Nevertheless Lindstrom et al. (2006) demonstrated that NuA4 (together with ISWI and Swr-C) plays a role in repression of stress induced genes.

1.3.1.2. SAGA:

SAGA contains the histone acetyltransferase subunit Gcn5 which predominantly modifies H2B and H3 (Zhang et al. 1998; Suka et al. 2001). The complex facilitates binding of TATA-binding protein (TBP) to the promoter and is recruited by an activator that interacts with its Tra1 subunit. At the Galactose- induced Gal1 promoter histone acetylation is observed before formation of PIC (Bhaumik and Green, 2001) and in contrast to transcription factor IID (TFIID, which binds to the core promoter) SAGA associates to upstream activating sequences (UAS) (Larschan and Winston, ,2001; Bhaumik and Green, 2001). While TFIID is involved in transcription of 90% of all yeast genes, SAGA is important for the remaining, including stress genes (Huisinga and Pugh, 2004).
Gcn5 and Esa1 both play a role in the acetylation of histone variant Htz1 at K14. These HATs have overlapping functions (Millar et al. 2006).

### 1.3.2. Methylation

1.3.2.1 Methylation of H3K4

The Set1 complex (also referred to as COMPASS) methylates H3K4 residues on the entire ORF region of actively transcribed genes. Whereas monomethylation patterns accumulate at the 3'end, dimethylation is found to be more pronounced in the middle of the genes and trimethylation is increased at the transcriptional start site. The "accumulation" of these modifications is dependent on the frequency of transcription and its exact function is currently

unknown. Studies indicate that H3K4 methylation is likely to work as a signal for factors involved in initiation/repression of transcription that can specifically recognize this modification.

1.3.2.2. Methylation of H3K36

Tri- and dimethylation by methylase Set2 are enriched in the 3' ORF region. H3K36 trimethylation leads to recruitment of Rpd3S histone deacetylase (HDAC) complex. The chromodomain Eaf3 of Rpd3S is able to recognize H3K36 methylation and the deacetylase removes acetyl- residues on H3 (Figure I4). Hyperacetylation within an ORF caused by disruption of the Set2-Rpd3 pathway appears to enable the underlying DNA sequence to become exposed to TFs, thus enabling cryptic promoter-like sequences within the ORF to function as transcription start sites (Carrozza et al., 2005; Joshi and Struhl, 2005) which lead to shortened aberrant transcripts.

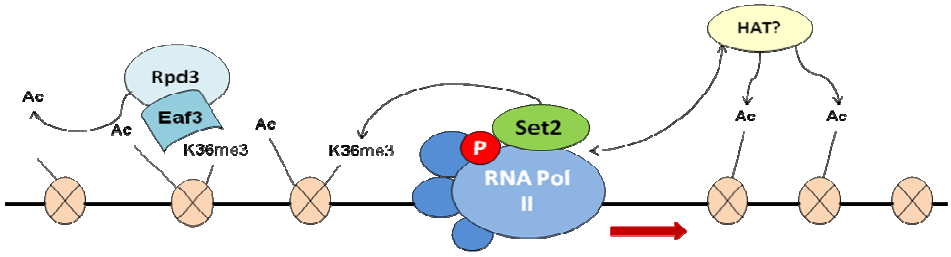



**Figure I4** Histones are acetylated by a HAT. During elongation the Set2 methylase methylates K36 on Histone 3. This modification is recognized by the chromodomain Eaf3 of the Histone- deacetylase Rpd3S. HDAC activity leads to hypoacetylated environment. (Adapted from Li et al. Cell 128, 707–719, February 23, 2007 [a]2007 Elsevier Inc.)

### 1.3.3. Ubiquitination of H2B

Ubiquitination of H2B is a stimulating signal for methylation of H3K4 and enhances the rate of transcription elongation. The activity of Rad6, the enzyme that ubiquitinates histones, is dependent on the activity of the PAF multisubunit complex and active transcription. Ubiquitination stimulates di- and trimethylation of histone H3K4 by Set1 (COMPASS) (Dehe et al., 2005; Shahbazian et al., 2005; Sun and Allis, 2002).

## 1.4. Histone Chaperones

To make sure that histones properly assemble into nucleosomes they are bound by histone chaperones which generally exhibit preference for H2A/H2B or H3/H4 dimers. Histone chaperones play a role in initiation but more importantly in elongation of transcription.

### 1.4.1. Asf1 (Anti- silencing factor 1)

Schwabish and Struhl, (2006) have shown that the histone chaperone Asf1 is not only associated with promoter but also with coding regions of actively transcribed genes. Asf1 is a multifunctional histone chaperone involved in initiation (as shown for the promoters of Pho5 and Pho8, Adkins et al 2004) and also in elongation interacting with the PAF1 elongation complex. In *asf1*Δ mutants the actively transcribed (i.e induced) *GAL10* and *GAL1* genes, have decreased levels of Pol II and increased levels of H3. Thus Asf1 is likely to be involved in eviction of H3 and H4 (as they are assembled in dimers) to facilitate travelling of Pol II but also helps reassembling of histone octameres (see Figure I5). In addition *asf1*Δ mutants display increased transcription from cryptic promoters within open reading frames indicating that regions with unassembled or poorly assembled nucleosomes allow Pol II to inappropriately initiate transcription. Because yeast *asf1*Δ mutant cells did not show any dramatic changes in nucleosome density (Adkins and Tyler 2004) it was proposed that other factors substitute in the reassembly process of histones.

### 1.4.2. Spt6

Spt6 interacts with H3 and assembles nucleosomes in vitro. Mutation of Spt6 leads to initiation from cryptic promoters (Kaplan et al., 2003), suggesting that this chaperone is involved in reassembling of nucleosomes behind traveling PolII. Promoter nucleosome reassembly mediated by the histone chaperone Spt6 is required for transcriptional repression of Pho5, Pho8, Adh2, Ady2 and Suc2. When Spt6 is inactivated by mutation, chromatin reassembly at promoter regions is impaired and transcription initiation occurs independent of transcriptional activators (Adkins et al. 2006). Furthermore, Spt6 is also responsible for histone reassembly after transcriptional elongation in the ORF regions of Pho5. Mutants of Spt6 show increased levels of RNA Pol ll on the ORF of Pho5.

### 1.4.3. FACT (facilitates chromatin transcription)

FACT is required by Pol II to transcribe through chromatin and interacts, like Asf1, with the elongation factor Paf1. However not every gene seems to be dependent on this histone chaperone complex. Studies showed that FACT was capable of facilitating elongation through some nucleosomes but not others. The requirement of FACT could be dependent on the positioning of the nucleosomes and also on the strength of histone- DNA interaction.

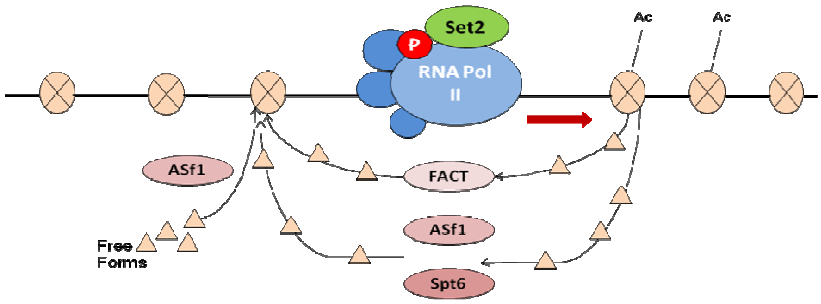



**Figure I5** The histone chaperones FACT, Asf1 and Spt6 facilitate disassembly and reassembly of histones during passage of elongating RNA Pol II. (Adapted from Li et al. Cell 128, 707–719, February 23, 2007 ª2007 Elsevier Inc.)

## 1.5. Chromatin Remodeling Complexes

### 1.5.1. ATP-dependent Chromatin-Remodeling Complexes

Chromatin remodelling complexes are a class of factors implicated in changing chromatin structure. ATP- dependent chromatin remodeling factors alter the contacts between nucleosomes and DNA (Becker et al. 2002; Tsukiyama et al., 2002; Emerson et al. , 2002; Narlikar et al., 2002; Katsani et al., 2003; Korber et al., W. Horz et al., 2004 Mizuguchi et al., 2004) by several mechanisms:

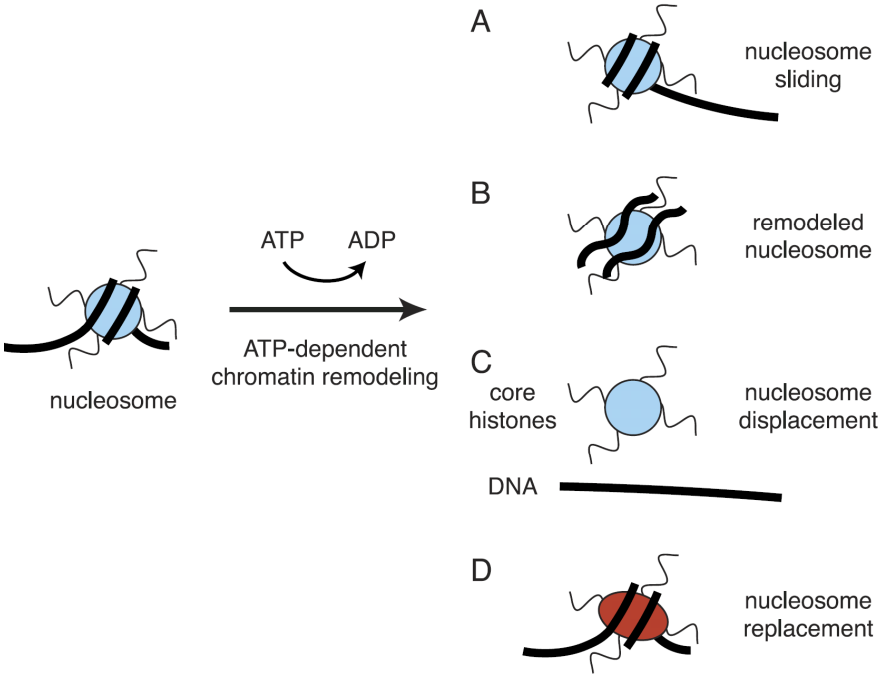



**Figure I6** . Mechanisms of Chromatin remodeling complexes (from L. Mohrmann, C.P. Verrijzer / Biochimica et Biophysica Acta 1681 (2005) 60)

(A) Nucleosome sliding, meaning that the position of a nucleosome on the DNA strand changes

(B) The creation of a state, where DNA becomes more accessible but histones remain bound

(C) The complete dissociation of DNA and histones

(D) Histone replacement with a variant histone

These mechanisms enable association of factors involved in initiation and elongation of transcription. ATP-dependent remodeling complexes work together with other factors such as histone chaperones and histone modifying enzymes such as histone acetylases (e.g. NuA4) and methylases (Set2). In budding yeast the superfamily SF2 of DEAD/H-box nucleic acid stimulated ATPases (Eisen et al., 1995) is the most prominent one. *S. cerevisiae* has five subfamilies of SF2: Swi2/SNF2 types (SWI, RSC), ISWI types (Iswi1, 2), CHD- types, Ino80-C types (Swr-C, Ino80-C) and YFR038w- types (in plants: DDM1) (reviewed in van Vugt, 2007).

### 1.5.1.1. Swi2/Snf2

| | |
|---|---|
| **ySWI/SNF** | - non-essential for viability<br>- low abundance<br>- facilitates expression of several inducible genes<br>- involved in exit from mitosis through stimulation of expression of some mitotic genes<br>- implicated in transcriptional activation and repression |

**Table 2** (taken from L. Mohrmann, C.P. Verrijzer / Biochimica et Biophysica Acta 1681 (2005) 67)

The ySwi/Snf complex was the first described chromatin-remodeling complex, contains 12 subunits and is not essential for viability. Furthermore only 200 copies of the complex exist in a cell making it not very abundant. Yeast cells contain two closely related Swi/Snf-type remodeling complexes with distinct cellular functions, ySwi/Snf itself and RSC (remodel the structure of chromatin).

Homologous complexes of the Swi/Snf family of remodelers have been identified in Drosophila and mammals, thus these complexes are highly conserved. Swi/Snf is able to cause a loss of H2A- DNA interaction and furthermore nucleosomes partially unwrap at promoter regions because of Swi/Snf activity (Kassabov et al., 2003; Lorch et al. 2001; Flaus et al., 2003). This activity facilitates efficient access of transcription factors (Schwabish and Struhl, 2007).

### 1.5.1.2. RSC

| | |
|---|---|
| **RSC** | - essential for viability<br>- highly abundant<br>- target genes distinct from those of ySWI/SNF<br>- involved in transcriptional activation and repression<br>- Sth1, Sfh1, Rsc3 and Rsc9 are essential for cell cycle progression through G2/M<br>- involved in sister chromatid cohesion and segregation |

**Table 3** (taken from L. Mohrmann, C.P. Verrijzer / Biochimica et Biophysica Acta 1681 (2005) 67)

A yeast chromatin remodeling complex closely related to Swi/Snf is RSC (Remodel the Structure of Chromatin) (Cairns et al., 1994; Cairns et al. 1999). Similar to Ino80-C, RSC contains 17 subunits. In contrast to ySwi/Snf, RSC function is required for yeast viability. Indicative of broader roles in chromatin dynamics, RSC is very abundant, while Swi/Snf is not (10-fold). This notion is reflected by recent studies that have implicated RSC in sister chromatid cohesion, transcription regulation (Angus-Hill, 2001), chromosome segregation and DNA repair (Hsu et al., 2003; Baetz et al., 2004; Huang et al., 2004; Wong et al., 2002) In the context of transcription RSC is suggested to collaborate with the histone chaperones Spt6 and Nap1 in a reaction coupled to histone acetyltransferase activity (Carey et al., 2006). A process stimulated by the HATs NuA4 and SAGA enables RSC to help RNA- PolII through the nucleosome barrier indicating that histone acetylation recruits RSC (Carey et al., 2006).

### 1.5.1.3. ISWI

The budding yeast *S. cerevisiae* has two members of ISWI ATPases: Isw1 and Isw2 (Tsukiyama et al., 1999). The function of Isw2 is to slide nucleosomes to repress the transcription of several classes of genes in parallel with the Sin3/ Rpd3 histone deacetylase (Fazzio et al., 2001; Fazzio et al., 2003; Goldmark et al., 2000). Isw1 was shown to be involved in repression of stress genes working together with Swr-C and NuA4 (Lindstrom et al., 2006). Thus the three complexes have parallel activities for regulation of stress response genes.

### 1.5.1.4. SWR-C

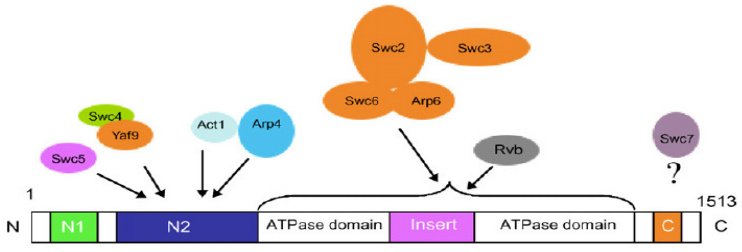



**Figure I7** Subunits of Swr-C (taken from Y. Bao, X. Shen, Ino80-C subfamily of chromatin remodeling complexes, Mutat. Res.: Fundam.
Mol. Mech. Mutagen. (2007), 2)

The purified yeast Swr-C complex contains fourteen polypeptides which are partly shared with Ino80-C. The Swr-C complex is capable of specifically exchanging histone H2A in nucleosomes for its variant Htz1 and thus Swr-C and Htz1 share a common function in transcription regulation. Swr1 is a Swi2/Snf2-related ATPase with a split conserved ATPase

domain, characteristic of the core ATPases of the Ino80-C subfamily. Similar to the Ino80 complex, the Swr-C complex exhibits ATPase activity (Mizuguchi et al., 2004).

The major function of the Swr-C complex is to deposit the histone variant dimer Htz1– H2B into nucleosomes by replacing the pre-existing H2A– H2B dimer. The substitution of core histones with the corresponding histone variants can generate a structurally and functionally distinct region in the chromatin (Henikoff et al., 2004; Chakravarthy et al., 2004). In contrast to canonical core histones, histone variants are synthesized outside of S- phase and their incorporation into the chromatin structure is independent on DNA replication. The histone H2A variant, H2AZ (in yeast Htz1), is highly conserved from yeast to man. Depending on its acetylation status Htz1 is found at inactive promoters (when not acetylated). The depositioning is also dependent on the activation potential of the promoter (Guillemette et al. 2005; Li et al.2005; Zhang et al. 2005; Millar et al. 2006).

It was found that NuA4 and Swr-C mutants share similar phenotypes and that the complexes interact genetically, suggesting important functional links between them (Kobor et al., 2004). Similar to the Ino80-C complex the Swr-C complex may also play a role in DNA repair. The Swr-C complex, and its associated H2AZ deposition activity, may be recruited to DSBs, in the concert with NuA4 and/or Ino80-C complexes, to exchange γ-H2AX (H2A phosphorylated at Ser129) with Htz1, which may further alter the local chromatin structure and facilitate the process of DNA repair.

### 1.5.1.5. The Chromatin Remodeling Complex Ino80

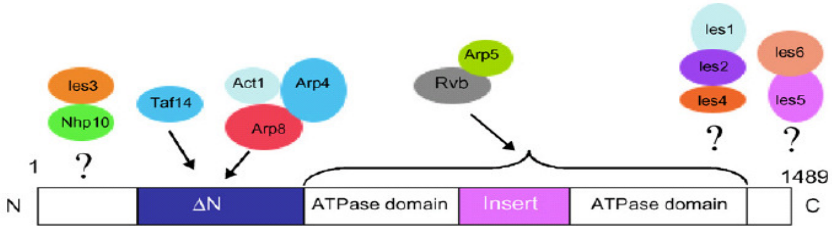



**Figure I8** Subunits of Ino80-C (taken from Y. Bao, X. Shen, INO80 subfamily of chromatin remodeling complexes, Mutat. Res.: Fundam. Mol. Mech. Mutagen. (2007), 2)

The ATP- dependent Ino80 remodeling complex is a member of the SNF2 subfamilies (Swi/Snf, ISWI, CHD, INO80 and YFR038 types). Ino80-C is a highly conserved complex containing 17 subunits, among them: Ino80, Rvb1, Rvb2 (Helicase activity), Arp4 (actin-related protein 4), Arp5, Arp8, actin, Nhp10 (non histoneprotein 10), Anc1/Taf14, and several Ino eighty subunits (Ies). The most prominent feature of the Ino80 ATPase domain is the presence of a spacer.

The Ino80 subunit is the core ATPase of the complex and also the main scaffold for the other subunits. Actin and actin-related proteins (Arps) have been identified as subunits in many chromatin modifying complexes (Boyer et al., 2000). Ino80-C contains conventional actin, Arp4, Arp5 and Arp8. Arp5 and Arp8 have been found exclusively in Ino80-C (Shen et al. 2000). The phenotypes of *arp5Δ* and *arp8Δ* are similar to that of *ino80*Δ thus Arp5 and Arp8 seem to be important for the chromatin remodeling function of Ino80. Crucially Arp4 and Arp8 show association to histones in vitro (Arp4 to H3 and H4) which suggests that both proteins could have some histone chaperone function.

Nhp10 has also been described as a subunit of Ino80-C. Ino80-C lacking Nhp10 has reduced DNA binding activity but is still capable of mobilizing nucleosomes which suggests that Nhp10 has, compared to the Arps, a less important role in chromatin remodeling.

Microarray data indicate that mutants of Ino80 display a 2-fold change in mRNA levels of about 150 yeast genes. Further studies have shown that the Ino80 complex regulates a specific set of genes both negatively and positively (van Attikum et al., 2004). The phosphate regulated genes *PHO5* and *PHO84* act as models for studying the mechanism of promoter regulation by Ino80-C. Ino80-C had a repressive effect on the *PHO5* promoter and an activating effect on the *PHO84* promoter. Furthermore, *in vitro* transcription assays show a 10- fold increase in transcriptional activation of chromatin remodeled by Ino80-C (Shen 2000 et al.). These data suggest the Ino80-C complex is involved in regulation of the expression of several genes in different contexts with different roles.

The Ino80 complex is not only involved in transcriptional regulation but has also been shown to be involved in DNA damage responses. Histone modifications and chromatin remodeling are important for the repair of DNA lesions, such as double strand breaks (DSBs). Phosphorylation of Serine 129 of H2A is crucial modification during the repair of DSBs. Furthermore, H2AX deficiency results in genomic instability. Phosphorylated H2AX (γ-H2AX), is essential for the association of several DNA repair proteins (Downs et al., 2000). In yeast, γ-H2AX is also required for the recruitment of the chromatin remodeling complex Ino80 to DSBs. This feature established a connection between chromatin remodeling and repair of DNA lesions (Downs et al., 2004, Morrison et al., 2007; van Attikum et al., 2004). γ-H2AX is necessary for association of Ino80-C to DSBs. The Nhp10 subunit of Ino80-C is most likely responsible for the recruitment of the complex to the phosphorylated histone. Interestingly, in the absence of actin, Arp4 and Arp8, the Ino80-*arp8*Δ-C mutant complex displayed significantly higher levels of interaction with γ-H2AX than that of the Ino80-*nhp10*Δ-C mutant complex, in which Nhp10 and Ies3 are lost, suggesting that Nhp10 and Ies3 play a more significant role in the interaction between Ino80-C and γ-H2AX than the Arps (Shen et al.2000).

# 2. Aim of this Work

In 2003 Görzer et al. described a phenotype of a mutant in the Actin related protein 4 gene (*ARP4*). This temperature sensitive mutant *(arp4$^{G161D}$)* had increased levels of stress genes transcripts that were dependent on the transcription factors Msn2 and Msn4. Arp4 is an functional essential subunit of the histone acetyltransferase NuA4 and the chromatin remodeling complexes Swr-C and Ino80-C. Shen et al. (2000) identified the Ino80 complex and using microarrays they found that Ino80 is involved in the repression of stress gene. Therefore, we suspected that Ino80-C was the candidate activity responsible for the *arp4* phenotype on stress gene mRNA levels.

In course of this work we investigated the cause of the *arp4* phenotype and define a role of Ino80-C in the repression of stress induced genes.

The primary questions addressed can be formulated as follows:

1) What is the cause of the *arp4$^{G161D}$* phenotype of hyperinduced stress genes?
2) In which of the three complexes (NuA4, Swr-C, Ino80- C) Arp4 function is involved in stress gene transcription?
3) What is the function and of the corresponding complex?

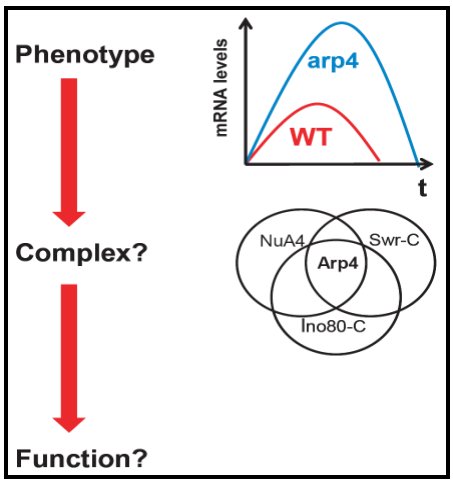



**Figure AW1** mRNA levels of stress response genes are hyperinduced in arp4ts mutants. Arp4 (Actin related protein 4) is a subunit of 3 complexes all involved in stress gene transcription.

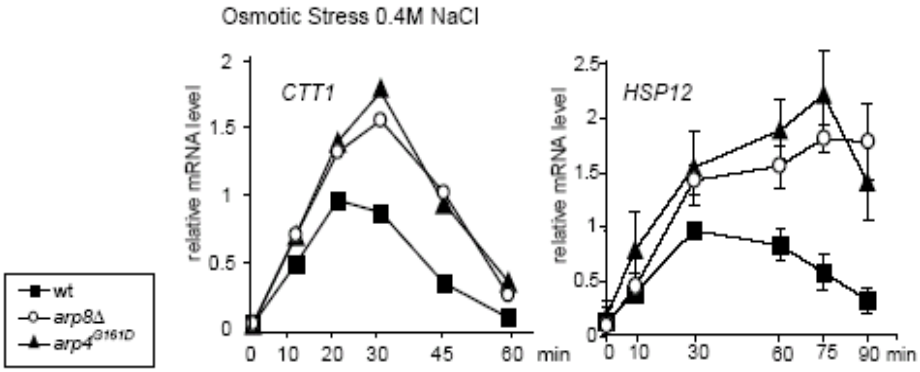
# 3. Results

## 3.1. Mutations in Arp4 and Arp8 confer sustained Stress Gene Expression.

Mutants compromising the function of the chromatin remodeling complex Ino80 such as Arp4 and Arp8, display enhanced levels of genes inducible by various types of environmental stress (Görzer et al., 2003; Shen et al., 2003b; van Attikum et al., 2004). A more detailed analysis of the expression of two genes highly induced by hyperosmotic stress (*CTT1*, *HSP12*) revealed increased and sustained mRNA levels in strains lacking Arp8 (*arp8*Δ) and in mutants carrying a temperature sensitive allele of Arp4 ($arp4^{G161D}$) (Figure 1A). Arp4 is a subunit also present in NuA4 and Swr-C complexes both implicated in increased expression of stress regulated genes (Lindstrom et al., 2006). To determine the contribution of these complexes to the observed increased transcription levels, we compared *CTT1* and *HSP12* expression in strains lacking specific subunits of all three Arp4-containing complexes such as *arp8*Δ (Ino80), *eaf5*Δ (NuA4) and *swr1*Δ (Swr-C). *CTT1* and *HSP12* mRNA levels were determined during osmostress and heat stress in these mutants (Figure 1B). mRNA levels from several experiments were quantified and normalized relative to highest wild type RNA level with *PPI1* as internal control. The resulting data demonstrate that among these mutants the absence of the Ino80 specific subunit Arp8 causes the most dramatic changes of transcript levels. The global contribution of Ino80 on stress induced transcription was analyzed by microarray transcript profile analysis of osmostress (0.4M NaCl) treated wild type and *arp8*Δ cells. After 30 minutes exposure to hyperosmolarity conditions, wild type and *arp8*Δ cells had a very similar transcript profile. However, at the 60 minutes time point we find globally increased transcript levels in the *arp8*Δ mutant of induced gene, whereas repressed genes were approximately unchanged. Transcript levels of stressed (0.4M NaCl, 60 minutes) wild type and *arp8*Δ cells were also compared directly on microarrays. In the *arp8*Δ mutant we find an approximately 1.5 fold mean increase of the RNA level of those genes which are more than two fold induced in the wild type. These data suggest a global function for Ino80 during stress induced transcription.

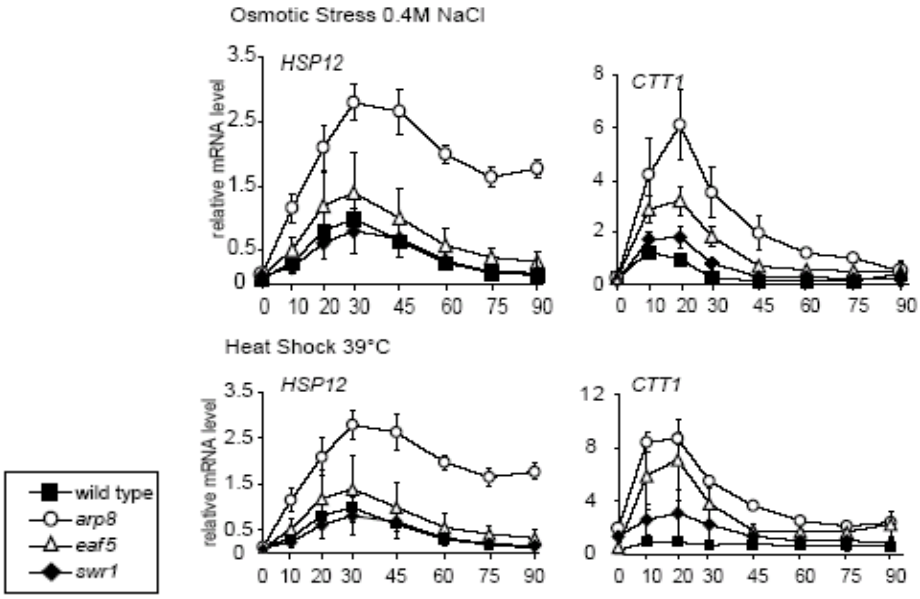
**Fig.1**

**A**



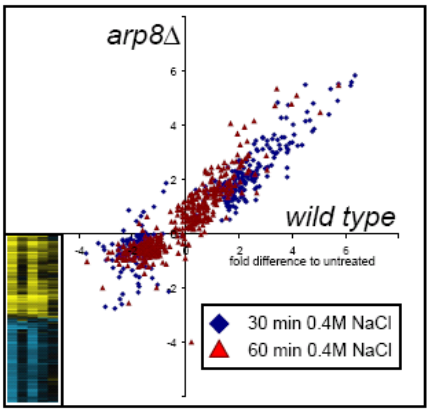

Stress Genes *CTT1* and *HSP12* are hyperinduced in *W303arp4G161D* and *W303arp8Δ*

**B**




Deletion of Ino80-C subunit arp8 causes the strongest effect on expression of *CTT1* and *HSP12* after osmotic and heat stress compared to mutants of NuA4 and Swr-C

**C**




Microarray analysis of *arp8*Δ mutant cells treated with 0.4M NaCl

**Figure 1.** Sustained transcript levels in *arp8*Δ and *arp4*$^{G161D}$ mutants. (**A**) mRNA levels of *CTT1* and *HSP12* during osmostress (0.4M NaCl) are up regulated in *arp8*Δ and *arp4*$^{G161D}$ strains. Wild type (W303-1A) and mutant strains *arp4*$^{G161D}$ (LK100) and *arp8*Δ (LK236) were grown in YPD at room temperature (RT) and stressed with 0.4M NaCl for the indicated times. (**B**) Northern analysis of *CTT1* and *HSP12* mRNA levels during osmostress and heat stress in *arp8*Δ, eaf5Δ and swr1Δ mutants (BY4713 derivatives). mRNA levels were quantified relative to highest wild type level, loading was normalized to *PPI1*, values from several blots were averaged. (**C***) Global delay of down regulation of transcripts in osmostress treated *arp8*Δ mutant cells. Microarray profile analysis of 0.4M NaCl treated wild type and *arp8*Δ cells at 30 and 60 minutes.

*Microarrays were performed in collaboration with the FH-Biotech

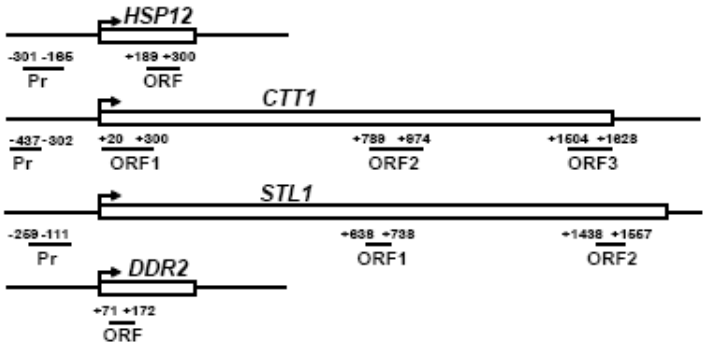
### 3.2. Arp4 and Ino80 are recruited to the ORFs of Stress Genes

The HSP12 gene is hyperinduced in mutants of Arp4 and Arp8 compared to wild type. To investigate whether Ino80-C is directly involved in the observed transcriptional changes we tested the recruitment of Arp4 to highly induced stress gene *HSP12* during heat stress by chromatin immunoprecipitation (ChIP) followed by quantification by PCR. The location of the PCR fragments of the analyzed genes is indicated in Figure 2A. The stress unresponsive gene *VCX1* was used as internal control throughout. A shift from 25℃ to 39℃ resulted in rapid recruitment of Arp4 to ORF and promoter region of *HSP12* (Figure 2B). The signal decreased rapidly after relieving stress conditions by shift to 25℃ (Figure 2B) suggesting a direct link between Arp4 recruitment and stress triggered transcription. To further investigate this recruitment we analyzed the kinetics of Arp4 association during heat stress (shift from 25℃ to 39℃) similar to the previous experiment. ChIP precipitates obtained with Arp4

antiserum were analyzed by PCR detecting promoter and ORF regions of *CTT1, HSP12* and *DDR2* (Figure 2C left panels). Recruitment of Arp4 to the promoter of *DDR2* was not detected (data not shown) in contrast to a strong recruitment to the ORF of this gene. From the same extracts Ino80-HA was precipitated using HA- antibodies. We found rapid association (after 4 minutes detectable!) of Arp4 and Ino80 to the ORFs of *CTT1, HSP12* and *DDR2* which was sustained for about twenty minutes (Figure 2C right panels). To determine whether Arp4 and Ino80 recruitment occurs also under different stress conditions we tested recruitment of both proteins after hyperosmolarity stress (0.4M NaCl). We included *STL1* which is a gene exclusively induced by osmostress. Analysis of the obtained PCR fragments showed increased signals of both Arp4 and Ino80-HA 6- 10 minutes after treatment. Recruitment of Arp4 and Ino80-HA had almost identical characteristics and was more pronounced to the ORFs of *CTT1, STL1* and *HSP12* compared to their promoter regions. These data support a direct role of the Ino80 complex for stress gene transcription and indicate a strong recruitment to the stress gene open reading frames.

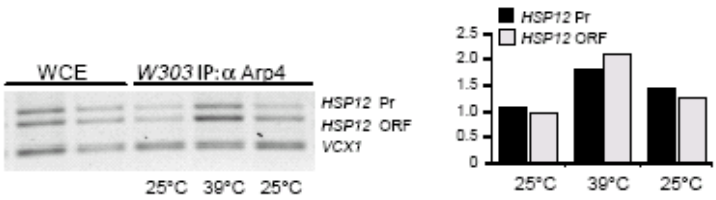
**Fig.2**

**A**



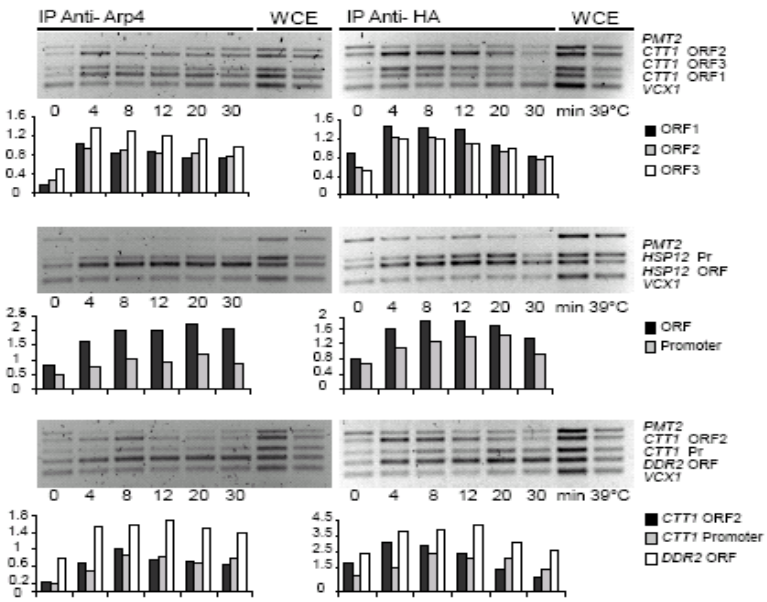

Promoter and ORF regions of *HSP12, CTT1, STL1* and *DDR2* detected in ChIP experiments

**B**



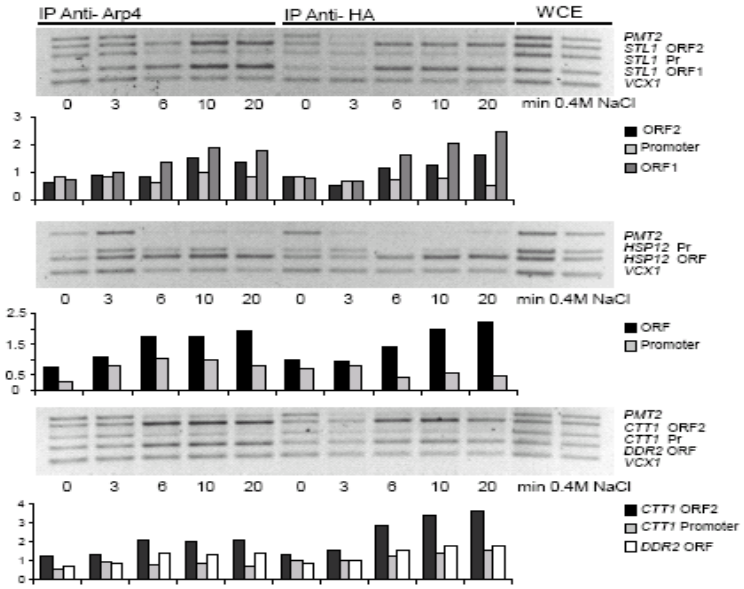

Arp4 is recruited to promoter and ORF regions of *HSP12* after heat shock. When stress is diminished Arp4 signal disappears

**C**




Both Arp4 (left panel) and Ino80-HA (right panel) are recruited to stress gene regions after heat shock with similar kinetics

**D**




Both Arp4 (left panel) and Ino80-HA (right panel) are recruited to stress gene regions after osmotic stress with similar kinetics

**Figure 2.** Arp4 and Ino80 recruitment to promoter and open reading frame regions of activated stress genes. (**A**) Arp4 is transiently recruited to the *HSP12* gene during heat shock. W303-1A cells were was grown at 25℃, shifted to 39℃ for 10 minutes and then shifted back. Chromatin immunoprecipitation assays (ChIP) were performed with Arp4-antibody. A region within the *VCX1* ORF was used as an internal control, WCE - whole cell

extract. (**B**) Kinetics of chromatin association of Arp4 and Ino80 after heat shock (25° to 39°C). Strain JW205 was grown at RT and shifted to 39°C for the indicated times. (**C**) Kinetics of association of Arp4 and Ino80 upon osmostress (0.4 M NaCl). Strain JW205 was grown at 25°C and exposed to osmotic stress (0.4M NaCl) and Ino80-HA chromatin association was determined at the indicated times. (**D**) Location of the primers used for ChIP experiments at *HSP12, CTT1, STL1* and *DDR2* loci.

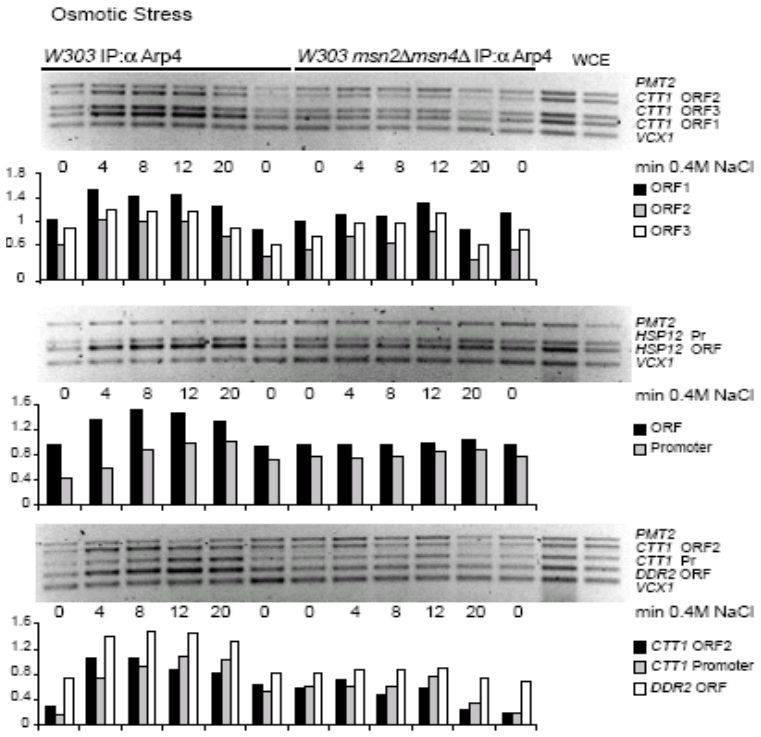
Experiments in Figure 2 were performed together with Ludmila Paskova

### 3.3. Arp4 and Ino80 Association requires Function of Transcription Factors

Many of the stress genes up-regulated in the arp4$^{G161D}$ and arp8Δ mutants are dependent on the general stress regulatory factors Msn2 and Msn4 (hereafter referred to as Msn2/4) (Görzer, 2003; van Attikum 2004). Consequently we examined whether Arp4 association to stress gene ORFs and promoters requires function of transcription factor Msn2/4. Wild type and *msn2*Δ*msn4*Δ cells were exposed to osmotic stress (0.4M NaCl) and Arp4 recruitment to *CTT1, HSP12,* and *DDR2* ORFs and promoters was detected as described above. Crucially no associated Arp4 could be discovered in the coding regions and promoters of these genes in *msn2*Δ*msn4*Δ cells (Figure 3A).To exclude a specific function (mediation of recruitment) of Msn2/4 we investigated the recruitment of Arp4 to *STL1*, a gene independent of Msn2/4 and under the control of the transcription factor Hot1. Whereas *CTT1* and *HSP12* are almost entirely regulated by Msn2/4, *STL1* is mainly regulated by Hot1. However all three gene require the function of MAP- kinase Hog1 for induction by hyperosmolarity. We could observe that the recruitment pattern of Arp4 to ORFs and promoters coincides with the described activation pattern. Absence of Hot1 and Msn2/4 prevented recruitment of Arp4 to *STL1* and CTT1, respectively. Importantly, Hog1 was required for Arp4 recruitment to both *CTT1* and *STL1* (Figure 3B). To examine whether stress conditions per se are sufficient for Arp4 and Ino80 recruitment we detected Arp4 and Ino80 association to ORF and promoter of *STL1*. The *STL1* locus coding for a glycerol/$H^+$ symporter is rapidly induced by osmotic stress but not by heat stress. Following ChIP Arp4 and Ino80 could be found to be associated to *STL1* ORF and promoter after osmotic stress but not after heat shock (Figure 3C). These data indicate that stress gene induction but not a specific kind of stress or transcription factor is important for Arp4 and Ino80 recruitment.

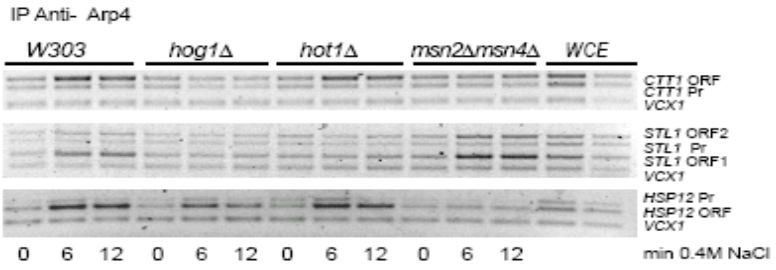
**Fig.3**

**A**




In the absence of transcription factors Msn2 and Msn4 Arp4 is not attracted

**B**




The presence of activating transcription factors is necessary for recruitment of Arp4

**C**

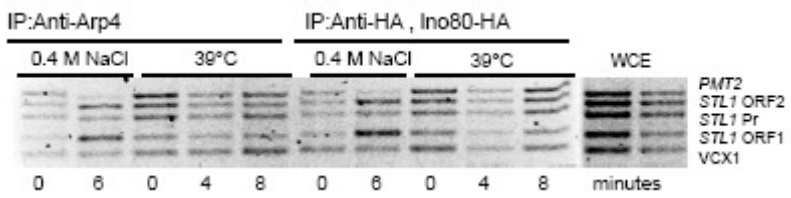


**Figure 3.** Ino80 and Arp4 are recruited to activated stress genes. (**A**) ChIP assay for Arp4 recruitment in wild type and *msn2*Δ*msn4*Δ strains after heat stress (25℃ to 39℃. (**B**) Osmostress induced recruitment of Arp4 to Hot1 and Msn2/4 regulated genes. Both *STL1* and *CTT1* require Hog1 for induction by osmotic stress. *WT*, *hog1*Δ, *hot1*Δ and *msn2*Δ*msn4*Δ strains were grown at 25℃ and treated with 0.4 M NaCl for 6 and 12 minutes or left untreated. (**C**) Ino80-HA and Arp4 recruitment to the *STL1* gene during salt stress and heat shock. Ino80-HA strain was grown at 25℃ and treated with 0,4 M NaCl or heat shocked to 39℃ for the indicated times. Arp4-Ab or Anti-HA (Ino80-HA) were used for ChIP. Precipitated DNA was analyzed by PCR of fragments indicated in Figure 2D Experiments in Figure 3 were performed together with Ludmila Paskova (A and C ) and Andriy Petrishyn (B)

### 3.4. Arp4 Association requires Elongation.

Arp4 and Ino80 recruitment could be a consequence of opening of the promoter chromatin of activated stress genes. However, recruitment to the distal end of the ORF suggested an involvement Pol II. To test if elongation by Pol II is required for Arp4 recruitment, we used a conditional mutant (*rpb1-1*) of the largest subunit of RNA Pol II (Nonet et al., 1987) which rapidly inactivates Rpb1 function upon shift to the restrictive temperature (37℃). Wild type (*RPB1*) and *rpb1-1* mutant strains were grown at the permissive temperature (26℃) and shifted to 37℃ for 1 hour to inactivate the *rpb1-1* allele and to adapt cells to higher temperature. Both cultures were then exposed to hyperosmolarity stress (0.4 M NaCl). Arp4 recruitment measured after 6 and 12 minutes was not influenced by temperature in the wild type (*RPB1*). Similarly, we observe Arp4 recruitment at the permissive temperature in the *rpb1-1* mutant strain. However, inactivation of RNA Pol II resulted in a significant decrease of the association of Arp4 with the ORF of *HSP12*, *CTT1* and prevented increase of recruitment to the *DDR2* ORF (Figure 4 A,B). Thus, it seems likely, that activity of RNA Pol II is required for recruitment of Arp4.

**Fig.4**

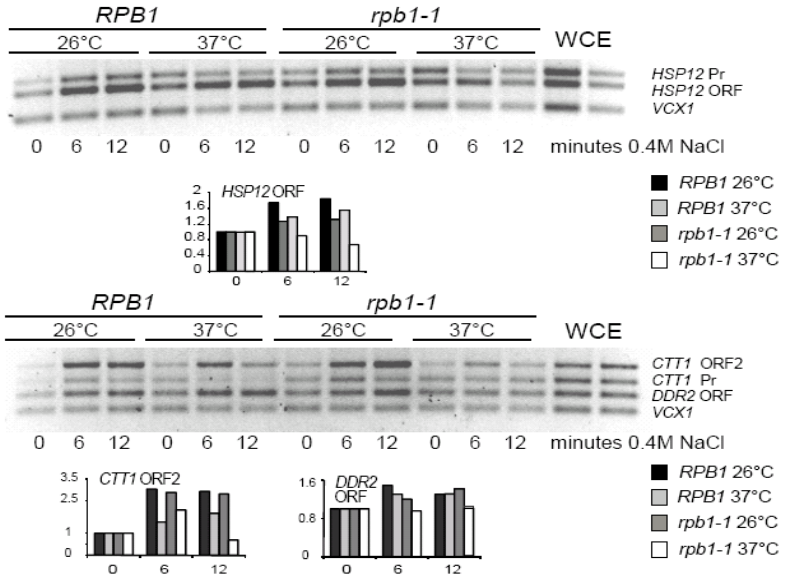



**Figure 4.** Inhibition of RNA Pol II prevents Arp4 recruitment. Wild type and *rpb1-1* strains were grown at the permissive temperature (26°C) one part shifted to 37°C for one hour to inactivate Rpb1. Both were then treated with to 0.4 M NaCl for 6 and 12 minutes. ChIP analysis of Arp4 recruitment to *HSP12*, *DDR2* and *CTT1* promoter and ORF regions. The relative amount of Arp4 occupancy normalized to VCX1 is indicated. Experiments in Figure 4 were performed together with Ludmila Paskova

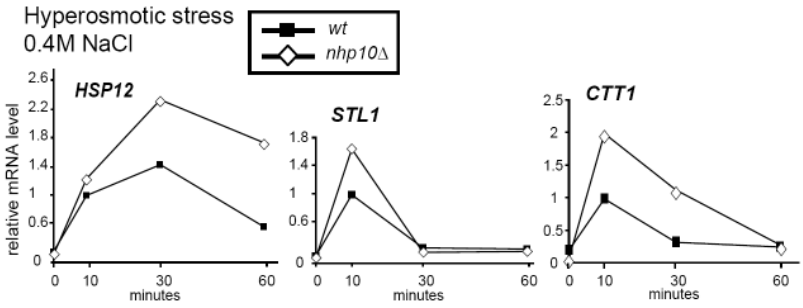
## 3.5. Recruitment of Ino80 to Stress Genes does not require Phosphorylation of H2A

The above results raised the question how Ino80-C is attracted to ORF regions of activated stress genes. One reported mechanism for recruitment of Ino80-C is during repair of double strand breaks (DSB) which is dependent on phosphorylation of S129 of H2A by the kinases Mec1 and Tel1 resulting in γH2A (van Attikum et al., 2004 Morrison et al., 2004 Papamichos-Chronakis et al., 2006 van Attikum et al., 2007 Morrison et al., 2007). The Ino80-C subunit Nhp10 is thought to interact directly with γ-H2A (Morrison, 2004 et al.) to recruit Ino80-C to DSBs. Thus we tested the effect of *NHP10* deletion on expression of stress genes. Interestingly, cells lacking Nhp10 show similar changes in *HSP12, STL1* and *CTT1* expression after hyperosmotic stress as mutants with compromised Ino80 function (*arp8*Δ, *arp4ts*) (Figure 5A). This result could indicate that recruitment of Ino80 to stress genes is mediated by a similar mechanism as during DSB repair. If this was the case interference with histone H2A S129 phosphorylation would also interfere with Ino80 recruitment during

expression of stress genes. Therefore we analyzed expression patterns of strains carrying H2A S129A mutant alleles of the two genes coding for H2A (hta1S129Ahta2S129A) and found that transript levels of *CTT1, HSP12* and *STL1* were not significantly increased after hyperosmotic stress (Figure 5B). This result suggests that Ino80 recruitment does not require γH2A. To demonstrate this directly we examined whether Ino80 could associate to stress gene ORFs in the hta1S129Ahta2S129A mutant strain by ChIP analysis. Association of Ino80-HA precipitated from salt- stressed wild type and hta1S129Ahta2S129A showed a similar kinetics in both strains (Figure 5C). This result suggests that enhanced stress gene expression in *nhp*Δ mutant is not based on a γH2A linked Ino80 recruitment defect and suggest that phosphorylation of H2A serine 129 is not an essential signal for recruitment of Ino80 to ORF regions of stress induced genes.

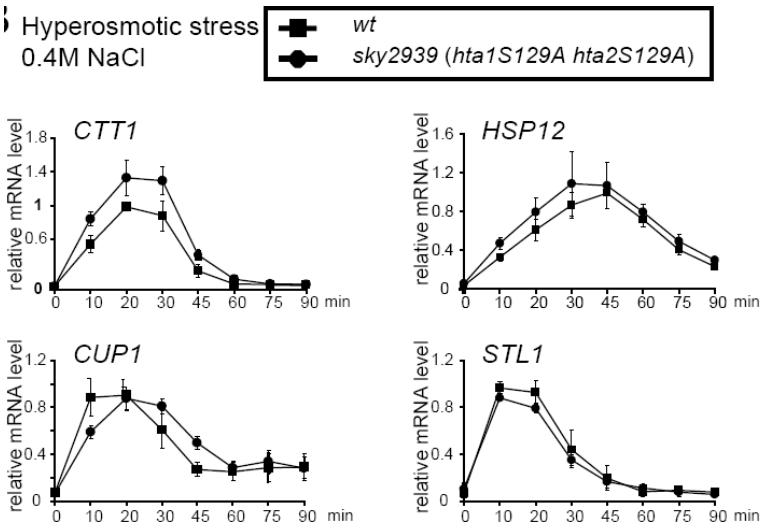
**Fig 5**

**A**



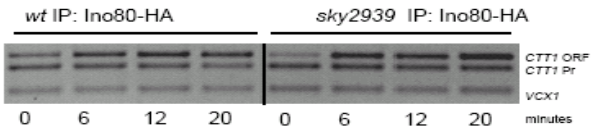

Absence of Ino80 subunit Nhp10 leads to similar expression effects as mutation of Arp4 and lacking of Arp8

**B**



Mutation of H2A phosphorylation site S129 does not significantly affect expression of CTT1, STL1, HSP12 and CUP1

**C**




Phosphorylation of S129 does not attract Ino80-HA to CTT1 ORF and promoter

**Figure 5.** INO80 recruitment to stress gene ORFs does not require γ-H2A. (**A**) Absence of *NHP10* subunit of INO80 increases of the mRNA level of *HSP12*, *STL1* and *CTT1*. Wild type and *nhp10*Δ strains were grown at 30°C and exposed to osmotic stress (0.4M NaCl). Quantification of northern blots is shown. (**C**) Absence of phosphorylation in H2A S129A mutants does not cause major changes of mRNA levels of stress genes. Wild type and *sky2939* (*hta1S129A hta2S129A*) strains were grown at 30°C and exposed to osmotic stress (0.4M NaCl) for *CTT1*, *HSP12*, *STL1* or treated with 100µM $Cu^{2+}$ for *CUP1* for the indicated times. (**D**) Absence of phosphorylation of H2A S129 is not required for recruitment of Ino80 to *CTT1* and *STL1* ORF regions. ChIP analysis at the indicated time points with a chromosomal HA tagged Ino80 derivative.

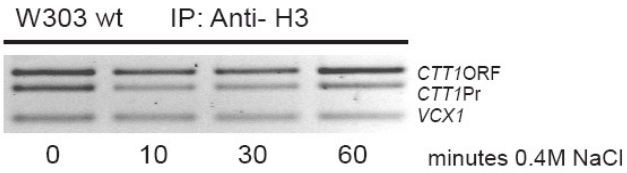
## 3.6. Histone Deposition from *STL1* and *CTT1* is delayed in *arp4$^{G161D}$* mutant Cells

Since Ino80-C has chromatin remodeling activity, we tested if it has a role for histone density during stress gene induction. Loss of Ino80-C function was achieved by incubating *arp4$^{G161D}$* mutant cells for 2 hours at the restrictive temperature (37°C). Arp4 protein is largely eliminated by this treatment (Görzer et al., 2003). *STL1* and *CTT1* ORF and promoter regions were analyzed for histone H4 and H3 during hyperosmotic stress (Figure 6). Histone H3 and H4 levels were determined by ChIP following quantitative PCR analysis. Hyperosmotic stress resulted in rapid loss of H3 and H4 from ORF and promoter of *STL1* in both wild type and *arp4$^{G161D}$* mutant strains. Reassembly of histone H3 back onto *STL1* ORF and promoter region was severely delayed in the *arp4$^{G161D}$* mutant. While H3 occupancy of the *STL1* promoter and ORF reaches levels of untreated cells within 30 minutes after stress induction, in the *arp4$^{G161D}$* mutant only half of the pretreatment level was regained (Figure 6A, 6B). Histone H4 was evicted rapidly during stress from *CTT1* and *STL1* ORF and promoter regions. In the wild type deposition of H4 started immediately, while in *arp4$^{G161D}$* mutant this

process was delayed. The pattern of H2A occupancy was similar to H4 (data not shown). These results indicate that Arp4 is required for efficient reassembly of H3 and H4 after activation of transcription by osmotic stress.

**Fig 6**

**A**




Histone H3 disappears from ORF and promoter of stress gene *CTT1*

**B**

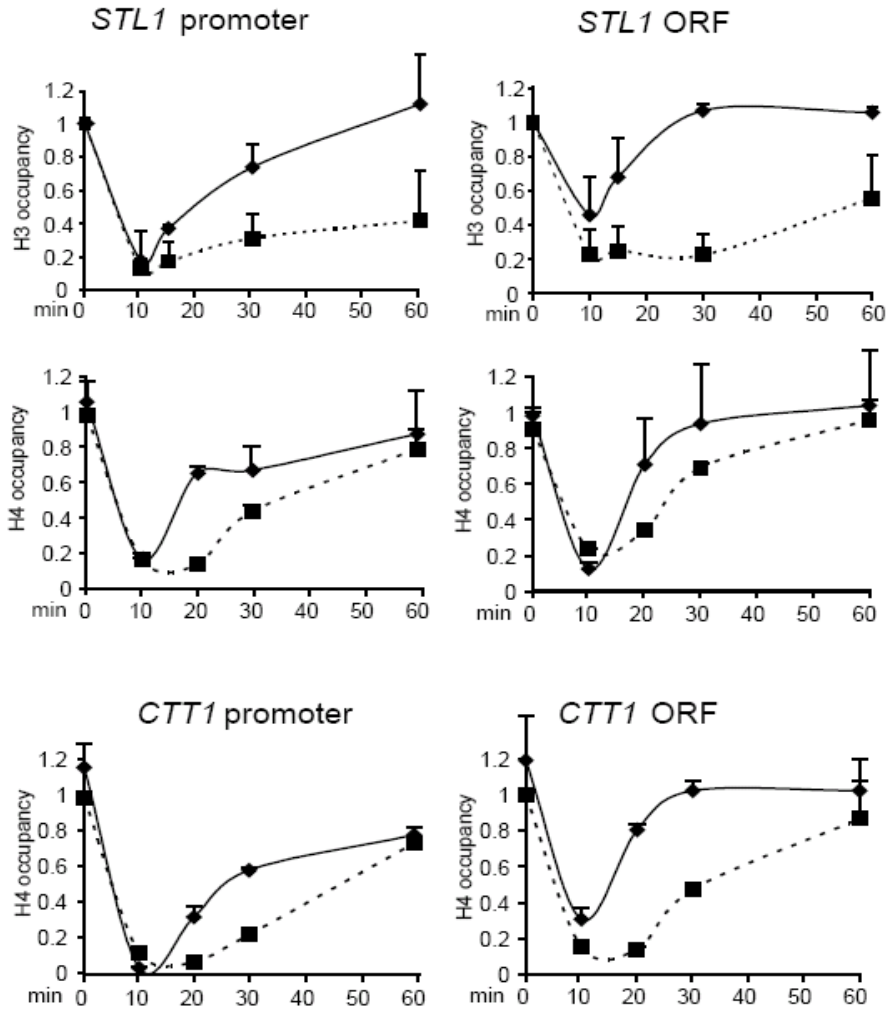


**Figure 6.** A) Histone H3 disappears from ORF and promoter of *CTT1*. Cells were stressed with 0.4M NaCl for times indicated and ChIP was performed as described before B*) Inhibition of Arp4 delays histone H3 and H4 reassembly. Wild type and *arp4G161D* strains were grown in YDP shifted to non-permissive temperature (37ºC, 2h 30 min) to inactivate the *arp4 ts* allele and treated with 0.4M NaCl for the indicated times. Histone H3 and H4 occupancy at the promoter and coding regions of *STL1* and H4 occupancy at the promoter and coding regions of *CTT1* was determined by ChIP and quantitative PCR as described.

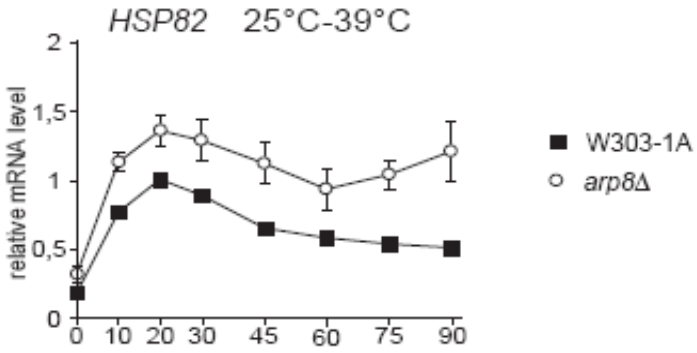
* Experiments in Figure 6B were performed by Glora Màs Cell signaling unit, Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra (UPF), Parc de Recerca Biomèdica de Barcelona (PRBB), Barcelona, Spain.

### 3.7. Rapid Activation of Transcription attracts Ino80

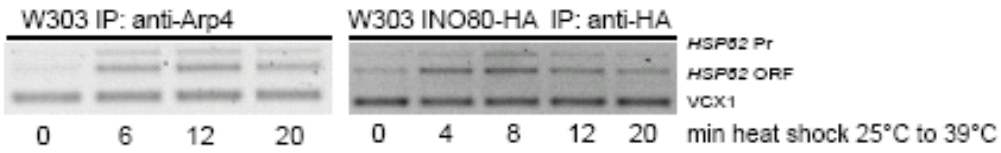
Stress induced genes have sustained and increased levels when Ino80-C function is compromised. This raises the question in which regulatory context Ino80-C function contributes to control of transcript levels. The *CTT1*, *HSP12,* and *STL1* genes analyzed so far are induced by heat and osmotic stress under the control of the transcription factors Msn2/4 and Hot1, respectively. Next we tested if Ino80-C function is also required for proper control of transcription mediated by other conditions and transcription factors. We found that heat stress induction of the Hsf1 regulated gene *HSP82* is enhanced and sustained in *arp8*Δ mutant cells (Figure 7A). Rapid recruitment of Arp4 and INO80 to the *HSP82* ORF suggests a direct role of Ino80-C for attenuation of transcription (Figure 7B). Furthermore, we analyzed copper stress as an entirely independent condition to the ones previously used. $Cu^{2+}$ ions trigger rapid activation of the transcription factor Cup2 leading to the almost immediate transcription of the *CUP1* gene. We find increased levels of *CUP1* transcript in $arp4^{G161D}$ mutant cells and a concomitant recruitment of Ino80-C to the *CUP1* ORF (Figure 7C, D). Hyperosmotic, heat, and copper stress activate transcription of the respective target genes within minutes and implicate Ino80-C function for proper control as shown above. In contrast, induction of *GAL10* transcript levels triggered by addition of galactose approach a high level after 30 minutes and reach a constant level after approximately 60 minutes which was not influenced by the absence of Arp8 as was the decline of *GAL10* message after repression of transcription by addition of glucose. These data suggest that compromised Ino80-C function affects genes which are rapidly induced.

**Fig.7**

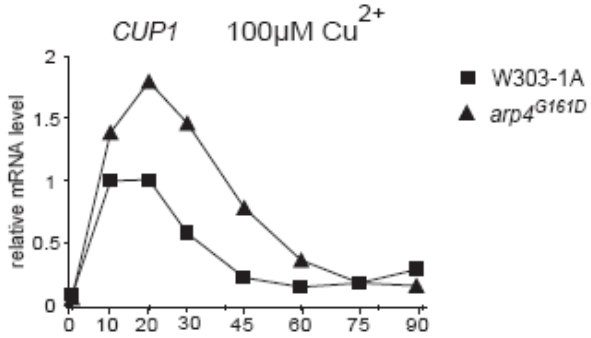
**A**


HSP82 25°C-39°C
relative mRNA level
2
1,5
1
0,5
0
0 10 20 30 45 60 75 90
W303-1A
arp8Δ

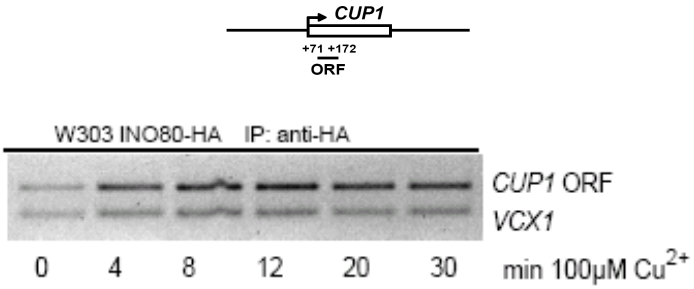
**B**


W303 IP: anti-Arp4
W303 INO80-HA IP: anti-HA
HSP82 Pr
HSP82 ORF
VCX1
0 6 12 20
0 4 8 12 20
min heat shock 25°C to 39°C

**C**


CUP1 100μM Cu2+
relative mRNA level
2
1.5
1
0.5
0
0 10 20 30 45 60 75 90
W303-1A
arp4G161D

**D**


CUP1
+71 +172
ORF
W303 INO80-HA IP: anti-HA
CUP1 ORF
VCX1
0 4 8 12 20 30
min 100μM Cu2+

**E**

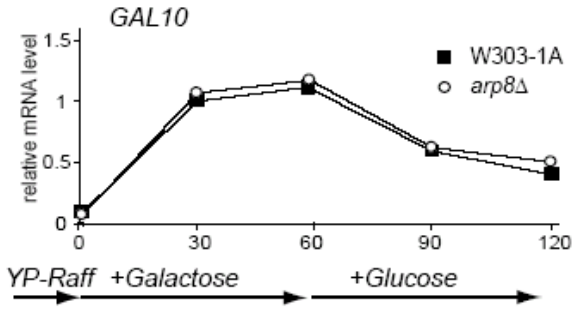



**Figure 7.** INO80 functions generally during attenuation of stress gene transcription. (**A**) *HSP82* mRNA levels in w*ild type* and *arp8*Δ mutant strain after shift to 39ºC for the indicated times. (**B**) Heat shock induced recruitment of Arp4 (IP anti-Arp4) and Ino80 (IP anti-HA) to *HSP82* promoter and ORF. **(C)** Expression of *CUP1* following treatment with 100µM $Cu^{2+}$ in wild type and *arp8*Δ mutant strain. **(D)** Recruitment of Ino80-HA to the *CUP1* ORF during induction by copper treatment. **(E)** Arp8 absence does not change mRNA level of *GAL10*. *WT* and *arp8*Δ strains were grown in YP medium supplemented with Raffinose. Galactose was added to induce *GAL10* expression and after 60 minutes glucose was added to repress *GAL10* expression.

### 3.8. Mutants of Asf1 and Set2 show Hyperinduction of Stress Genes

From the previous experiments we learned that Ino80-C is involved in depositioning of histones after osmotic stress (Figure 6). From microarray experiments of Zabaronick and Tyler (2005) we knew that strains lacking Asf1 (*asf1*Δ) displayed increased transcription levels of environmentally induced genes. Therefore we wanted to check whether *asf1*Δ had similar kinetics of stress gene induction as Ino80-C mutants. Furthermore the elongation coupled histone H3 methylase Set2 is involved in repression of transcription by indirectly activating the histone deacetylase Rpd3S. Thus we also wanted to test whether compromised Set2 function would result in prolonged and increased expression of stress genes. In the wild type strain HSP12 transcripts reach their maximum levels 30 minutes after induction with 0.4 M NaCl and are nearly not detectable after 90 minutes. In the *asf1*Δ strain mRNA levels decrease much slower compared to wt. After 90 minutes HSP12 mRNA is still detectable in significantly higher amounts compared to wt. 60 minutes after stress induction the *set2*Δ strain also displays increased transcript levels of HSP12 (Figure 8). These phenotypes have a similar character as Ino80-C mutants. These results indicate that Asf1, Set2 and Ino80-C might be involved in the same process.

**Fig. 8**

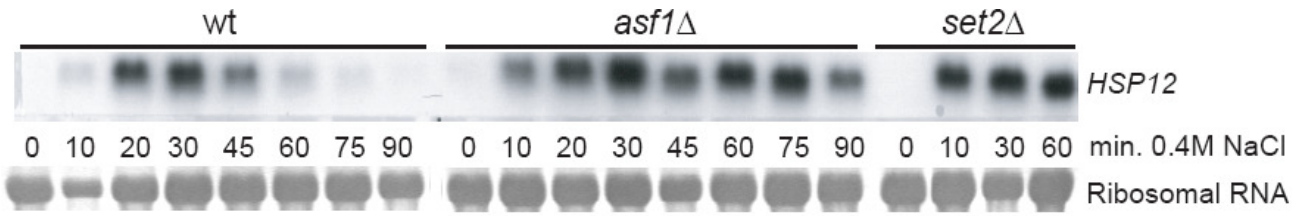



**Figure 8** Strains lacking Asf1 and Set2 show prolonged expression of Hsp12. Cells were stressed with 0.4M NaCl. Northern Blot was performed as described before. As “internal standards” ribosomal RNA bands are indicated.

# 4. Discussion

## 4.1. Overview

The results presented above give an insight into the role of the chromatin remodeling complex Ino80 during transcription of stress induced genes. The main results can be summarized as follows:

- The phenotype of the *arp4*$^{G161D}$ mutant is a consequence of compromised Ino80-C function and not of compromised NuA4 or Swr-C
- Mutants compromising Ino80-C (*arp8*Δ, *arp4*$^{G161D}$, *nhp10*Δ) increase levels of stress gene transcripts and in many cases also lead to sustained expression kinetics.
- Association of Ino80-C requires the function of active transcription factors and elongation
- Ino80-C is involved in reinstalling chromatin structure after/during passage of RNA Pol II
- Asf1 and Set2 mutants display similar phenotypes as Ino80-C mutants. Ino80-C recruitment is independent on phosphorylated γH2A.

## 4.2. The Role of Chromatin Remodeling and modifying Complexes for Stress Gene Transcription

Environmental stress transiently induces the transcription of many genes (Gasch et al.; 2000). Transcripts induced by cellular stress show a rapid increase but also a rather fast decrease to avoid overproduction of protecting proteins and waste of resources. Thus many stress genes are in a repressed state allowing quick transcriptional changes. The fast conversion of a repressor to an activator allows fast association of coactivators like SAGA and Swi/Snf to promoter regions. Stress genes, in contrast to house keeping genes, are mostly dependent on the HAT activity of SAGA and the presence of TATA elements (Basehoar et al. 2004; Huisinga and Pugh, 2004). Thus the control of gene expression is in part dependent on the basic structure of their promoters. However, there are many factors which contribute to regulation in the context of individual loci. These include the type, number and spacing of transcription factor binding site, the location within the nucleus or the position along the chromosome (Basehoar et al. 2004; Huisinga, 2004).

A certain level of general repression is established by chromatin structure (e.g. nucleosome packing). Several reports have shown that expression of environmentally regulated genes is connected with chromatin remodeling and thus with chromatin remodeling

complexes and chromatin modifications. The chromatin remodeling complex RSC, for example, has been shown to be involved in stress gene activation whereas ISWI, NuA4 and Swr-C have an impact on repression. Strains lacking combinations of subunits of these three complexes show increased mRNA- levels of genes regulated by environmental conditions (Lindstrom et al. 2006). Furthermore, the absence of the histone chaperone Asf1 was also shown to cause increased expression levels of stress genes (Ohkuni et al., 2003; Zabaronick and Tyler, 2005).

### 4.3. The Function of Ino80-C: Reinstallation of Chromatin Structure

As already described above RNA polymerase II and associated factors displace all nucleosomes that form a barrier to elongation. All four histones are dynamically evicted and reassembled upon passage of Pol II through coding regions (Kristjuhan and Svejstrup, 2004; Lee et al., 2004; Zhang et al., 2005; Zhao et al., 2005). Our experiments have shown that histone eviction is not compromised by mutation of Ino80-C (to be more precise of the subunit Arp4). Crucially, the reassembly of H3 and H4 is significantly delayed.

The process of histone eviction and repositioning of histones is currently not well described but histone chaperones like Asf1, FACT (Mason and Struhl, 2003; Schwabish and Struhl, 2007) and Spt6 (Kaplan et al., 2003) are recruited by elongating Pol II to facilitate passage through the nucleosome barrier. They are also required to re-establish the proper chromatin structure behind travelling Pol II. Strains lacking Asf1 show significantly prolonged expression of stress genes (Zabaronick and Tyler, 2005 and Figure 8) similarly to Ino80-C mutants. Thus Asf1 and Ino80 could function in the same process. Asf1 reassembles H3 and H4 to establish repressed chromatin structure and Ino80-C may facilitate this process upstream or downstream of Asf1. Furthermore, Bao and Shen (2007) suggest a possible histone chaperone function of Ino80-C. Ino80-C could work as a histone chaperone and thus support function of Asf1 during transcription of very fast and highly transcribed genes. This hypothesis is supported by the fact that Asf1 is also recruited to actively transcribed genes (Schwabish and Struhl, 2006) similar to Ino80-C. Both factors could work together in repopulation of regions devoid of histones and therefore chromatin structure.

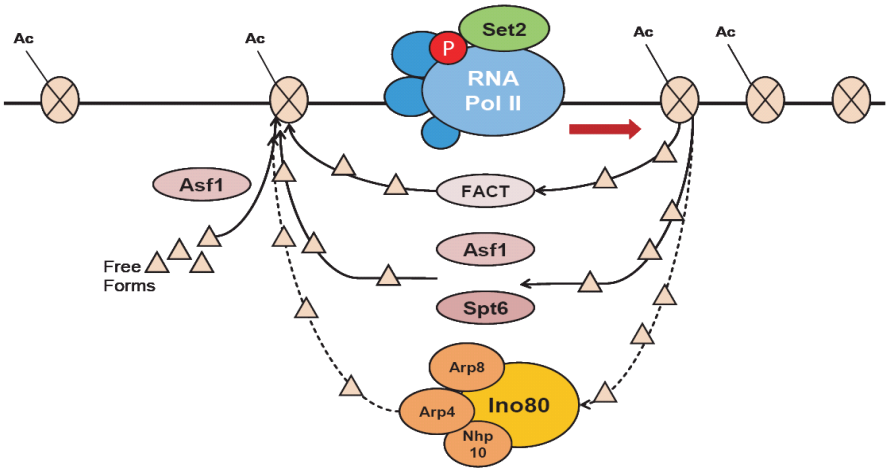



**Figure D1** Possible function of Ino80-C during transcription of very fast expressed genes. Ino80-C could support function of histone chaperone Asf1 (adapted from Li et al. Cell 128, 707–719, February 23, 2007 ª2007 Elsevier Inc.)

It is known that Ino80 has both ATPase and 3'-5' helicase activity (displayed by the subunits Rvb1 and Rvb2). In addition to its possible function as a histone chaperone during transcription of highly active genes Ino80-C could also contribute to reestablishment of repressive chromatin structure by nucleosome sliding, but the exact mechanism how this and other chromatin remodeling complexes function is not completely clear. Current models for nucleosome sliding and subunit replacement propose inter- and intranucleosomal DNA loop formation (Flaus and Owen-Hughes, 2004; Zhang et al., 2006; Zofall et al., 2006).

It has been reported that the Set2- Rpd3S pathway is involved in repression of transcription. The methylase Set2 is recruited by elongating Pol II and methylates Lysine 36 (K36) on Histone H3 (Trimethylation). This modification can be recognized by the Eaf3 chromodomain of the HDAC Rpd3S. Rpd3S removes Acetyl- residues on the histone tails. Removement of Acetylation leads to reestablishment of stable chromatin conformation. In case of unfrequently transcribed genes, lacking of Set2 leads to formation of aberrant transcripts. Deacetylation of histones but also redeposition of histones are required to maintain a stable conformation within ORFs and hinder aberrant binding of transcription factors and formation of PIC.

In stress gene transcription lacking of Set2 does not lead to cryptic transcript formation but to prolonged transcription (Figure 8). The phenotype of *set2*Δ mutants is similar to *asf1*Δ cells and mutants of Ino80-C. The kinetics of stress gene transcription is likely to be too fast to allow formation of cryptic transcripts.

Although deposition of histones and the deacetylation of histones by the Set2- Rpd3S pathway are actually both involved in establishment of a stable chromatin conformation, a direct connection between the two mechanisms has not been established yet. Carrozza et al.

(2005) propose that acetylation of histones could somehow facilitate interaction with histone chaperones. The redeposited histones can have two origins:

1) The preexisting histones are redeposited. In this case Rpd3S removes acetylation that occurred during elongation (RNA Pol II is associated with a HAT!)

2) Free forms of nucleosomes from the nucleus are also available

Set2 methylation may mark the newly reassembled histones for deacetylation and histone chaperones (possibly including Ino80-C) provide histones for the methylation by Set2 leading to chromatin conformations described above.

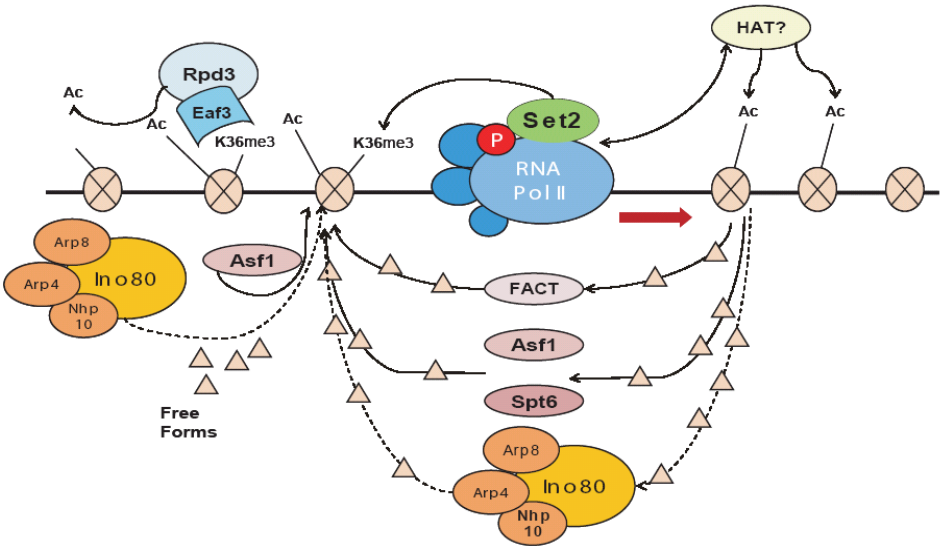



Figure D2 Possible involvement of Ino80-C in repression of transcription. Asf1 histone chaperone function is facilitated or supported by Ino80-C function (adapted from Li et al. Cell 128, 707–719, February 23, 2007 ª2007 Elsevier Inc.)

We propose that the Ino80 remodeling complex supports chromatin repopulation and thus repressive chromatin structure both preventing reinitiation and inhibiting elongation of stress genes. Ino80-C, as a relative abundant complex might be involved in a general surveillance mechanism detecting nucleosome deprived regions and restoring chromatin density by either sliding in nucleosomes from neighboring sequences or aiding deposition of new nucleosomes via chaperones such as Asf1. Since INO80 has orthologues in *Drosophila* (dINO80) and human (hINO80) (Cai et al., 2007; Jin et al., 2005; Klymenko et al., 2006) it will be interesting to investigate their roles for stress regulated transcription. While covalent modification might be decisive for recognition by proteins that influence chromatin dynamics and function (Jenuwein and Allis, 2001), remodeling by complexes such as Ino80-C has its role to reestablish the order of things.

## 4.4. How is Ino80 recruited to Stress Gene ORFs and Promoters?

Evidence exist that Ino80-C is recruited together with NuA4 and SWR to double strand breaks (van Attikum, 2004). The ATM- kinases Mec1/Tel are part of the first factors that are recruited to newly formed double strand breaks (they follow the MRX complex and yKu) (see Figure). The kinases are responsible for phosphorylation of Ser129 on histone 2A resulting in γ- H2A. This phosphorylated form of the histone is an attracting signal for Swr and Ino80 (van Attikum, 2004; Morrison, 2004).

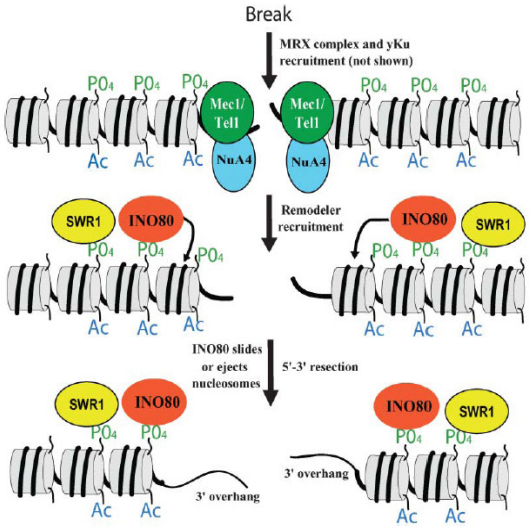



**Figure D3** Function of Ino80-C in DSB repair. Ino80-C is attracted by phosphorylation of Ser129 of H2A

During this study we investiated whether Ino80 is attracted by a similar signal to stress genes. Crucially Northern Blots and ChIP experiments (Fig5B and C) showed that γH2A does not play a role for the recruitment of Ino80. One important question is now what attracts the Ino80 complex to ORFs and promoters of stress genes. One possibility is that another histone modification such as methylation or ubiquitination is the signal for Ino80-C to attach to histones. Alternatively, decreased nucleosome density due to elongating RNA-Polymerase II could represent a recruiting signal as well as the polymerase itself. These models are to be addressed in the forthcoming work.

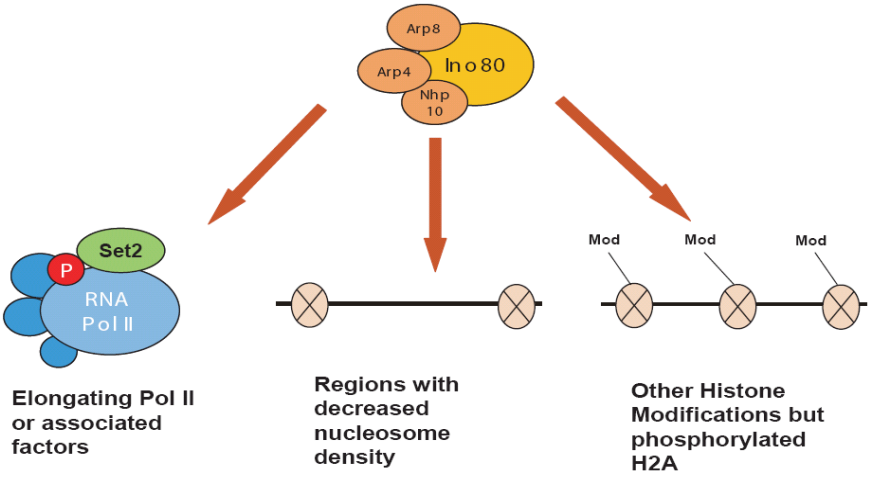



**Figure D4** Possible attraction signals for Ino80-C

# 5. Materials and Methods

## 5.1. Yeast Strains used in this Study

| Strains | Genotype | Reference |
|---|---|---|
| W303-1A | *MAT***a** *ura3-1 leu2-3,112 his3-11,15 trp1-1 ade2-1* | Nasmyth K. (Oxford) |
| W303-1B | *MAT*α isogenic to W303-1A | Nasmyth K. (Oxford) |
| LK100 | *MAT***a** arp4::*arp4G161D* (isogenic to W303-1A) | this study |
| LK236 | *MAT***a** *arp8*Δ::*His3-MX6* (isogenic to W303-1A) | this study |
| EK12 | *MAT***a** *INO80-9HA-KanMX4* (isogenic to W303-1A) | this study |
| JW205 | *MAT***a** *INO80-9HA-KanMX4* (isogenic to W303-1A) | this study |
| SKY2939 | *hta1S129A::his3MX6 hta2S129A::TRP1* (W303-1A) | {Downs, 2004} |
| BY4741 | *MAT***a***; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0* | {Brachmann, 1998} |
| BY*arp8*Δ | Y02397 YOR141c::kanMX4 (isogenic to BY4741) | euroscarf |
| BY*eaf5*Δ | Y00259 YEL018w::kanMX4 (isogenic to BY4741) | euroscarf |
| BY*swr1*Δ | Y03693 YDR334w::kanMX4 (isogenic to BY4741) | euroscarf |
| Y262 | *MATa ura3-52 his4-539 rpb1-1* | {Nonet, 1987} |
| Y233 | *MATa ura3-52 his4-539 RPB1:: URA3* | {Nonet, 1987} |
| BY *nhp10*Δ | *MAT***a** YDL002C::kanMX4 (isogenic to BY4741) | euroscarf |
| Sky 2939 Ino80- HA | *hta1S129A::his3MX6 hta2S129A::TRP1* (W303-1A) *INO80-9HA-KanMX4* | this study |
| UG43 | Matα *hot1::KanMX4* (isogenic to W303-1B) | Rep, 1999 |
| K327 | MATa *hog1::Trp1* (isogenic to W303-1A) | Rep, 1999 |
| W303 *msn2*Δ*msn4*Δ | MATa *msn2::HIS3 msn4::TRP1* | Görner 2002 |

**Table M1** Yeast strains used during this work

## 5.2. Methods

### 5.2.1. Northern Blotting

5.2.1.1. RNA Extraction (PCI Extraction)

Yeast cultures were grown to logarithmic phase (OD~0.7), stressed and the harvested for 2 minutes, at 2500rpm. The pellets were frozen in liquid nitrogen. Frozen cell samples were thawn on ice and resuspended in 200µl of RNA- extraction buffers. The samples were vortexed and glass beads (2/3 of total volume) were added. After vortexing again the samples were mixed with 200µl of phenol- chloroform- isoamylalcohol (PCI) solution. The yeast cells were broken using a "FastPrep", two times for 10 seconds, speed 6 and

afterwards centrifuged for fifteen minutes, full speed (4℃).The upper aqueous phase was taken off (180-190µl) and an equal amount of chloroform- isoamylalcohol (CI) solution was added. The two phases were mixed by inverting and then separated by 10 minutes of centrifugation at 4℃ full speed.The procedure was repeated (130-140µl off upper layer, equal amount of CI) then 120µl were taken off and 1/20 volume of 4M Sodium Acetate and two volumes of absolute ethanol were added. For precipitation the samples were put on -20℃ for up to one hour. Afterwards the tubes were centrifuged for 10 minutes, full speed in the cold room and after removing the supernatant the RNA pellets were washed with 80% ethanol (2 min. full speed, RT).The pellets were dried at 37℃ for 20min and resuspended in 50-100µl of DEPC-water. The concentration was determined as follows:
RNA solutions were diluted 1:400 in water and measured at OD260/280 in spectrophotometer (Hitachi U- 2000). The amount of RNA was calculated by the formula: OD1 at 260= 40µg/ml. 15µg-20µg were loaded on 1.2% agarose gels.

5.2.1.2. Sample Preparation and Gel Electrophoresis:

Sample Preparation:

| | |
|---|---|
| RNA and DEPC- water | 4.5µl (containing 15-20µg total RNA) |
| 5x FGRB | 2 µl |
| Formaldehyde (37%) | 3.5µl |
| Formamide | 10µl |
| sum | 20µl |

The samples were incubated on 62℃ for 15 minutes followed by quick spin. 2µl of DEPC-treated Loading buffer were added to each sample. The gels were run at max. 70V for five to six hours.

5.2.1.3. Capillary Blotting:

Three Whatman papers were put into a blotting apparatur filled wth 20x SSC. The agarose gel was put on the Whatman papers and the transfer membrane (Hybond™, Amersham) was added. The membrane was fixed with parafilm strips. And three Whatman papers incubated in 20X SSC were put on the sandwich followed by a pack of paper towels and glass plates. Glass bottles were used for reaching weight. (see Figure below)

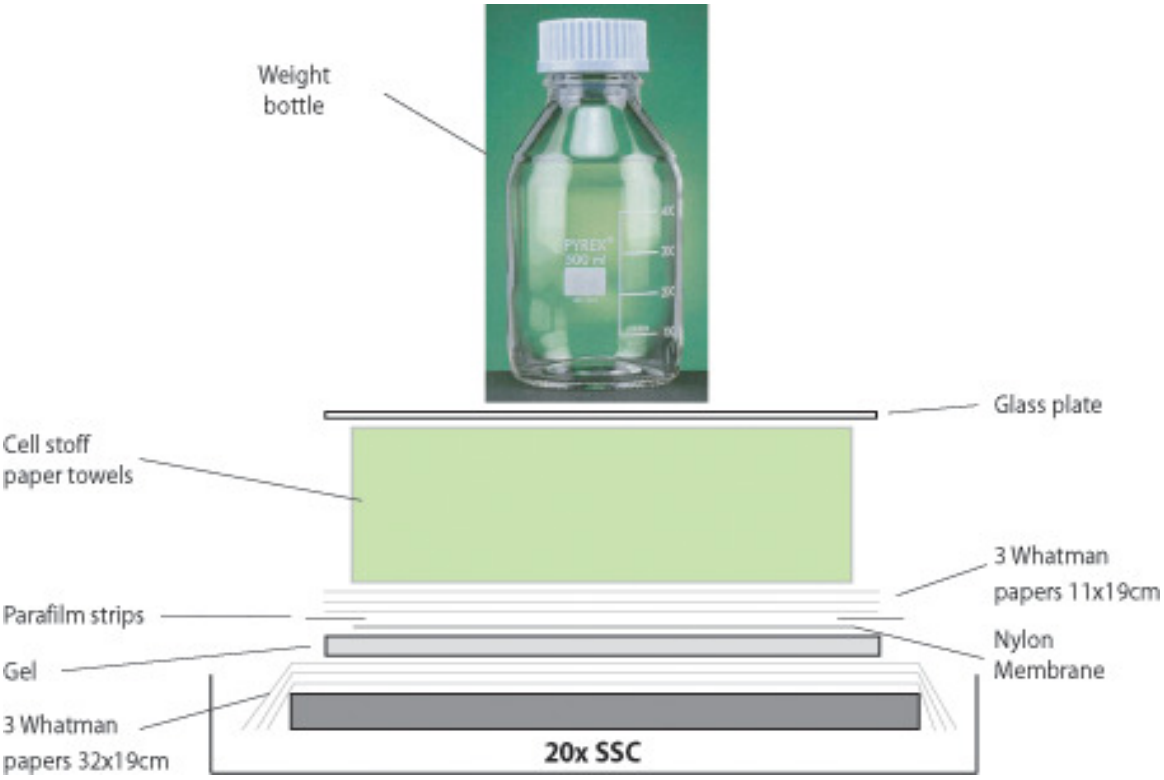



**Figure M1** Composition of capillary blotting equipment

5.2.1.4. Crosslinking, Dying and Prehybridisation

The Nylon membranes were crosslinked with 150kJ on both sides and then incubated with 10% Acetic Acid for 15 minutes. After removing the solution 5% Acetic Acid with Methylen Blue was added to dye ribosomal RNA and check for possible RNA degradation. The dye was removed with water (up to a few hours). Afterwards the membranes were put into glass bottles filled with 25ml of prehybridisation buffer and incubated on 63°C (in a hybridisation oven) for minimum 2hrs up to 2 days.

5.2.1.5. Preparation of Probes and Hybridisation

Amplification of Northern Blot Probes by PCR:

| | | |
|---|---|---|
| PCR- Mix: 50µl: | Taq- Polymerase | 2µl |
| | 10x Buffer (Invitrogen) | 5µl |
| | 50mM $MgSO_4$ | 1.5µl |
| | 2mM dNTPs | 5µl |
| | Primer for (10pMol/µl) | 2µl |
| | Primer rev (10pMol/µl) | 2µl |

| | |
|---|---|
| Genomic DNA (W3031A) | 2µl |
| $H_2O$ | 30.5µl |

Program: Robocycler (Stratagene) 45 cycles

| | | | | |
|---|---|---|---|---|
| 93°C | 93°C | 54°C | 72°C | 93°C |
| 5min | 1min | 1min | 1min | 5min |

PCR- products were checked on a 1% Agarose gel (Running Buffer 1xTAE) at 95-110V. Correct bands were cut and eluted from the gel by using Elution kit from Qiagen™.

Labeling of the probes with alphaP32dATPs using Prime-it 2 labeling Kit (Stratagene):

17.5µl of water together with the template (400ng) were mixed with 7µl of random primer 9mer and incubated on 95°C for five minutes. After quick spin 7µl of 5xdATP mix, 1µl Klenow polymerase and 2.5µl of alphaP32dATPs were added. The mixture was mixed by flicking (!) and incubated for one hour at 37°C.After 60µl of 1xTE were added the samples were loaded on a self- made sephadex column (G25, 6ml/g) to remove unincorporated nucleotides followed by centrifugation (2200rpm, 22sec).

Hybridisation, Washing and Detection:

The labelled probes were denaturated at 95°C for 2 minutes and 30 seconds, quickly spinned down and the desired volume was added to the membranes. Hybridization was performed over night. After removal of the hybridization buffer containing the radioactive probes the membranes were washed four times: Two times at room temperature for 15 minutes with washing solution and two times for fifteen minutes on 63°C. The washed membranes were wrapped into plastic bags and the signals were detected by X- ray at -80°C or room temperature for a few hours or over night depending on strength of the signal. Furthermore the membranes were also put into a PhosphoImager cassette for quantification.

### 5.2.2. Chromatin Immunoprecipitation

5.2.2.1. Incubation of Dynabeads with Antibody

During growth of the cell culture Dynabeads (Invitrogen) were incubated with antibody. The amount of beads and antibody were dependent on the characteristics of the antibody and were optimized.

| Antibody | Incubation time of | Amount of Antibody | Amount of Dynabeads | Time of IP |
|---|---|---|---|---|
| **Anti- HA** | ~ 6hrs | 8µl | 30µl | 1.5hrs |
| **Anti- Arp4** | ~ 6hrs | 4.5µl | 50µl | over night |
| **Anti- H2A** | over night | 8µl | 50µl | over night |
| **Anti-phospho-** | over night | 8µl | 50µl | over night |
| **Anti- H3** | over night | 8µl | 50µl | over night |

**Table M3:** Used Antibodies, incubation time with beads and time of IP

Before adding the antibody the beads were washed three times with 1x PBS/BSA. Then the beginning amount of PBS/ BSA was added. The washing step was repeated after incubation time and the beads were incubated in the beginning volume.

5.2.2.2. Harvesting of Cells and Whole Cell Extract

Over night cultures, 50ml per sample were grown to early logarithmic phase (OD ~0,500). After stress (heat shock, salt stress, copper) the cells were shaken with 1,4ml Formaldehyde (end concentration 1%) for 10-20min (dependent on Antibody) for cross- linking. The flasks were cooled down at 4℃ for approximately 1hr or over night following incubation with 2,5ml 2,5MGlycin for 10 minutes to stop crosslinking reaction. The cells were centrifuged at 2500 for 2 minutes, the supernatant was discarded and the pellets were washed four times in precooled falcon tubes with ice cold 1xTBS/PBS. The supernatant was removed completely and the pellet was resuspended in 600µl Lysis Buffer and put into an Eppendorf tube. Precooled glass beads were added (1/3 of liquid) and the tubes were vortexed for 7min, full speed. To make sure that enough cells were broken up the lysate was checked under the microscope.By using a needle a hole was made into the bottom of the sample tubes and they were stuck on the top of a fresh tube. Then the tubes were put into ice-cooled 50ml Falcon tubes and centrifuged for 2 minutes, 2200rpm. After sonification 4 times 15sec the samples were spinned for 10 minutes full speed in the cold room and the supernatants containing DNA were transferred to fresh tubes.10µl of the extract were saved as a control (whole cell extract, input).

5.2.2.3. Immunoprecipitation, Reversal of Cross-linking

Immunoprecipitation:

Time of Immunoprecipitation was dependent on the antibody (see above)

Washing of IP and Elution:

Immunoprecipitations were washed two times with Lysis Buffer, two times with Lysis Buffer 500mM NaCl, one time with Wash Buffer and two times with 1xTE, one ml each.DNA was eluted at 65°C with 50µl of 1xTE, 1%SDS, shaking hard for 10 min. Then 30µl of the supernatant were taken off and put into a fresh Eppendorf tube. Another 20µl of TES were added to the beads resulting in a total volume of 50µl again. The beads were again shaken for 10 min. and 30µl were taken off and added to the fresh tube already containing 30µl of eluate. To reach a total volume of 300µl, 240µl of 1xTES were added to the IPs (290 to Inputs!). The samples were incubated on 65°C for minimum 6hrs or over night. The samples were filled up to 600µl with 6µl of Glycogen, 15µl of Proteinase K (Merck) and 280µl of 1xTE. The mixes were incubated on 37°C for 1-1.5hrs.

DNA – Isolation and PCR:

600µl of PCI (cold) were added, the two phases were vortexed vigorously and separated again by 30 minutes of full speed centrifugation (cold room). The supernatant (570µl) was taken off and again 600µl of PCI were added following 20 minutes of centrifugation. As a last purification step 600µl of CI were added and the samples were mixed by flicking.The tubes were spinned full speed 5-10 min.In the meantime Eppendorf tubes were filled with 20µl of 5M NaCl. The upper aeqous phase was carefully taken off (500µl) brought together with the 5M NaCl and the DNA was precipitated by adding 600µl of Isopropanol. The solution was put on -20°C for minimum 20 minutes. The precipitates were spinned down for 10-15min full speed, the supernatant was taken off and the tubes were spinned down for another minute to remove rests of liquid. The DNA pellet was eluted in 30µl of 1xTE and used for PCR.

Primer Sequences:

| | |
|---|---|
| STL1prfor1 | CTTTTCATTGGGGCATTCGTAA |
| STL1prrev1 | TAGTGTTCCTACCAAGTAGCGAGC |
| STL1ORF1for | CTCTCTTCCTGCTTGCTTTCATG |
| STL1ORF1rev | TAGTGTTCCTACCAAGTAGCGAGC |
| STL1ORF2for | AGTTTGGAGGAAATCGACATCATC |
| STL1ORF2rev | TAGGTGTCTTCTACTCTATCTTCACC |
| CTT1forP2 | AGAACCTCCGTTATCTCCATTCC |
| CTT1revP2 | CAATACGCAATTTCACCGCTTG |
| CTT1ORF1fw | TGGTGGTTGTAGACTGGAGTTCG |
| CTT1ORF1rev | AACAAGACCAGGACATTTGTAACC |
| CTT1ORF2fw | AATCAGGCAAAGCTGTTCACTC |
| CTT1ORF2rev | ACATTCTCCGTTAGGGTGATGG |
| CTT1ORF3fw | GTTCATAACGTTGTTTGCCACG |

| | |
|---|---|
| CTT1ORF3rev | GGAACTCCCAAGCATTCTGCA |
| HSP12for2 | CGCAAGCATTAATACAACCCA |
| HSP12rev2 | AGAAAGACCTTTCAGATCAAGAGG |
| HSP12ORFfor | CAAGGATAACGCTGAAGGTCAAG |
| HSP12ORFrev | GACACGACCGGAATATTCG |
| DDR2ORFfor1 | ACGCATCCAACACCACGAGTAAC |
| DDR2ORFrev1 | CCAAAGCACCAACAGCAGCA |
| VCX1forCHIP | TGCGTGTGCATCCCTACTGA |
| VCXrevCHIP | AAGTGGTCTTCCTTGCCATGA |
| PMT2ORF1fw | GACATCGAGTATTTGAAGCCAGG |
| PMT2ORF1rev | GTCAATGTGTGCAACTTCTCAGG |
| HSP82 prom fw | CTGGTTCTTTCGAGTTTCGC |
| HSP82 prom | GAAATGAGGAGGTCACAGATG |
| HSP82ORFfw | GGATGAAGACGACAAGAAACC |
| HSP82ORFrev | CAGATGGGTTTCTAGTCCAC |

**Table M4:** Primers used for ChIP- PCR

ChIP PCR:

| **B** | **1x** | **C** | **1x** | **D** | **1x** | **E** | **1x** | **H** | **1x** |
|---|---|---|---|---|---|---|---|---|---|
| Ex-Tag | 0,1 | Ex-Tag | 0,1 | Ex-Tag | 0,1 | Ex-Tag | 0,1 | Ex-Tag | 0,1 |
| 10xBuf | 2 | 10xBuf | 2 | 10xBuf | 2 | 10xBuf | 2 | 10xBuf | 2 |
| 2,5 mM dNTP | 2 | 2,5 mM | 2 | 2,5 mM dNTP | 2 | 2,5 mM | 2 | 2,5 mM | 2 |
| STL1 orf2 | 0.2 | HSP12 (2) | 0.51 | CTT1 orf | 1.15 | CUP1orf | 0.04 | HSP82 | 0.6 |
| STL1 P | 1 | HSP12 ORF | 0.3 | CTT1 P2 | 0.27 | VCX1 | 1.5 | HSP82 | 0.5 |
| STL1 orf1 | 0.68 | VCX1 | 1.2 | VCX1 | 0.6 | DNA | 2.5 | VCX1 | 0.55 |
| VCX1 | 0.35 | DNA | 2 | DNA | 2 | $H_2O$ | 12.36 | DNA | 2 |
| DNA | 2 | $H_2O$ | 12.1 | $H_2O$ | 11.88 | | | $H_2O$ | 12.2 |
| $H_2O$ | 11.6 | | | | | | | | |

**Table M5:** ChIP- PCR mixes

PCR products were checked on 2.3% Agarose gel with 1xTBE as running buffer. Gels were run at 55-70V.

### 5.2.3. Tagging Ino80 with Hemagglutinin (Ino80-HA)

5.2.3.1. PCR of Tagging Cassette (kanMX )

Primers:

Ino80- tag- up: GCA AAA GCA TAA GTC AAG ATG GAA TTA AGG AAG CGG CAA GTG CAT TGG CAT CCG GTT CTG CTG CTA GT

Ino80- tag- low: GAG GCA AGA AAC TCA ACC AAA AGC TGA AGC TAC CCA AGA CGA TGA TTT CGC CTC GAG GCC AGA AGA

Plasmid: GA 2255 (kanMX)

PCR program:

| **94°C** | **53°C** | **72°C** | |
|---|---|---|---|
| 4min. | 1 min. | 1.5min. | **1x** |
| 1 min. | 1 min. | 1.5min. | **31x** |
| 1 min. | 1 min. | 7min. | **1x** |

PCR- Mix:

| | |
|---|---|
| Water | 37µl |
| Buffer (self made) | 5µl |
| dNTPs (10µM) | 2µl |
| Template | 2µl |
| Taq- Polymerase (self made) | 2µl |
| Primer for (1:10) | 2µl |
| Primer rev (1:10) | 2µl |
| Sum | 52µl |

PCR product was precipitated using 3M Sodium acetate and absolute ethanol

5.2.3.2. High Efficiency Yeast Transformation

An over night culture of S. cerevisiae was grown to logarithmic phase and spinned down at 2500rpm, 2 minutes. The culture was washed with 25ml of $dH_2O$ (another centrifugation step) and the pellet was resuspended in 1ml of 100mM Lithium Acetate and transferred to a fresh Eppendorf tube. After spinning at top speed for 5 seconds the supernatant was removed and the pellet was again resuspended in 100mM Lithium Acetate (400µl). The suspension was vortexed and the 50µl samples were pipetted. The samples were spinned down again and the rests of the Lithium Acetate were removed. Every sample was resuspended in the Transformation Mix:

- 240µl of Polyethylenglycol (50%)
- 36µl of 1M Lithium Acetate50µl of ss-DNA (10mg/ml); boiled for 5 min. and then immediately put on ice

- 25µl of disruption cassette (or 5µl of plasmid DNA and 20µl of water)

The pellet was vortexed vigorously for about one minute and then the cells were put on 30°C for 30 min. (recovery) followed by 20 min. of heat shock on 42°C. The transformants were spinned down, resuspended in 200-400µl of $dH_2O$ and plated on selective medium.

5.2.3.3. Identification of Positive Clones by Western Blotting

Protein Extraction (Yeast Protein TCA Extraction):

An over night culture with a volume of 4ml (in YPD) was harvested by centrifugation for 1 minute, 4000rpm (RT) (Alternatively 25ml of cells with OD1 could be used). The supernatant was discarded and the rest of the liquid containing the cells was transferred to a 1.5ml Eppendorf tube. The rest of the liquid was removed by spinning for one minute, full speed (RT). The cells without supernatant were frozen in liquid nitrogen, thawn on ice and resuspended in 100µl of 2M NaOH and 5% Mercaptoethanol (Stock can be stored at 4°C). The mixture was incubated on ice for ten minutes to lyse cells following addition of 20µl of 50% Trichlor- acetic acid (TCA) and 30 seconds of vortexing. To precipitate the proteins the lysates were again incubated on ice for 10 minutes.After centrifugation for 3 minutes, 13000 rpm the pellet was washed with 1M Tris pH7.5 to neutralize TCA (3 min., 13000rpm). The pellet was resuspended in 50µl of SDS sample buffer and boiled for 5 minutes at 95°C. Afterwards another centrifugation step was performed and the supernatant containing the proteins was transferred to a fresh tube.

Gel Electrophoresis:

Protein extracts were separated on 7% Acrylamide gels, run at 20mA/gel.

5.2.3.2. Blotting and Detection

Blotting was performed with a three transfer buffer system for two hours, 60mA, -20mV per membrane.

| |
|---|
| **+**<br><br>6 Whatman papers in Cathode buffer<br><br>Gel<br><br>Nitrocellulose membrane<br><br>3 Whatman papers in Anode 2 buffer<br><br>5 Whatman papers in Anode 1 buffer<br><br>**-** |

The Blots were blocked for 1 hour or over night in 25% milk in TBST, washed three times 15 min. in 1x TBST. The primary antibody was Anti-HA 1:5000 in 1xTBST, incubation was performed over night or for one hour, the secondary antibody was Anti- mouse, incubation time 1 hour. After washing three times in 1xTBST the signals were detected using ECL Western Blotting Detection System (Amersham).

### 5.2.4. Microarray

5.2.4.1. RNA Isolation

RNA was isolated as described for Northern Blotting (5.2.1.1.)

5.2.4.2. Direct labelling protocoll from the University Health Network Microarray Centre

Reverse Transcription

Reaction Mix:

| | |
|---|---|
| 5x First Strand reaction buffer | 8µl |
| AncT primer (5' T20VN, 100pmol/µl) | 1.5µl |
| aNTP- dCTP (6.67mM each of dATP, dTTP, dGTP) | 3µl |
| 2mM dCTP | 1µl |
| 1mM Cyanine 3 or Cyanine 5-dCTP (NEN) | 1µl |
| 0.1M DTT | 4µl |
| RNA (10-20µg total RNA) | |
| Nuclease– free water | to40 µl |

The two labelling reactions were incubated for 5min. at 65℃, spinned down and incubated for another 5 min. on 42℃ (saved from light!). Then 2µl of Reverse Transcriptase (SuperScriptII, Invitrogen) were added and the mixtures were incubated for two hours at 42℃ protected from light.

The reaction was briefly centrifuged and placed on ice. Then the reaction was stopped by adding 4µl 50mM EDTA, (pH8) and 2µl 10N NaOH and incubation on 65℃ for 20min. Afterwards 4.5µl of 5M Acetic acid were added and the pH- value was calibrated (pH~ 7). Labelled cDNA was purified using CyScribe ™ GFX™ (Amersham) Purification columns. The resulting 60 µl volume was reduced to 5µl by using Speed Vac.

Hybridization:

To each 100µl DIG Easy Hyb solution (Roche) 10µl of transfer DNA (10mg/ml) were added and the mixture was incubated on 65℃ for 2 minutes and then cooled to room temperature. The hybridization solution was mixed with the labelled cDNA and incubated at 65℃ from 2minutes and cooled down to RT. Hybridized coverslips were put into hybridization chambers containing a small amount of DIG Easy Hyb solution at the bottom. The lid was placed onto the box and the box was wrapped with plastic wrap following incubation at 37℃ over night.

Washing:

The coverslip was removed by dipping the array in 1xSSC. The slide was placed into a staining rack and placed into a staining dish with fresh 1xSSC. Each slide was washed for 15min. at 50℃in clean slide staining boxes containing pre-warmed (50℃) 1x SSC/ 0.1% SDS. After the washings were complete the slides were rinsed twice in RT 1xSSC and then in 0.1x SSC. Spin slides dry at 600rpm for 5 minutes in a slide box lined with Whatman paper (or in a Falcon tube, 500rpm, 5 min.).

Detection and Analyzation:

Axon 4000B scanner (Molecular Devices), and analyzed and normalized by using Gene Pix Pro4.1 software (Axon).

## 5.3. Buffers and Media

### 5.3.1. Buffers

#### 5.3.1.1 Chromatin Immunoprecipitation

10x PBS (1l): 8g NaCl

0.2g KCl

1.44g $Na_2HPO_4$

0.24g $KH_2PO_4$

1x TBS (1l): 20ml 1MTris-HCl pH7.5

30ml 5M NaCl

ChIP Lysis Buffer:

| **Lysis Buffer** | | **1x** |
|---|---|---|
| Hepes 250mM | ml | 1 |
| 5M NaCl | ml | 0,14 |
| 0,5M EDTA pH8 | µl | 10 |
| Triton X100 | µl | 50 |
| Sodium Deoxycholate | g | 0,005 |
| 0,1M PMSF | µl | 50 |
| Complete Solution EDTA | ml | 0,1 |
| Benzamidine | g | 0,0015 |
| Water | ml | 3,6435 |
| sum | ml | 5 |

For 500mM Lysis Buffer 776µl 5M NaCl per 10ml Lysis Buffer were added

ChIP Washing Buffer:

| **Washing Buffer** | | **1x** |
|---|---|---|
| 1M Tris HCl pH8 | µl | 20 |
| 5M LiCl (in Tris-HCl pH8) | ml | 0,1 |
| 0,5M EDTA pH8 | µl | 4 |
| NP40 | µl | 10 |
| Sodium Deoxycholate | g | 0,01 |
| Water | ml | 1,856 |
| sum | ml | 2 |

#### 5.3.1.2. Northern Blot

RNA Extraction Buffer: 50mM Tris- HCl pH 7-7.4

130mM NaCl

5mM EDTA

5% SDS

Phenol Chloroform Isoamylalcohol (PCI): 25:24:1 (Roti)

Chloroform Isoamylalcohol (CI): 24:1

DEPC- water: 0.1% Diethylpyrocarbonate (autoclaved)

Northern Blot Prehybridisation Buffer: 0.5M Sodium phosphate buffer pH7.2
7% SDS
1mM EDTA pH8

Washing Buffer: 0.5x SSC
0.1%SDS

Stripping Buffer: 1mM Tris-Cl pH8
1mM EDTA pH8
0.1x Denhardt`s reagent

Dying solution: 5% Acetic Acid
Methylenblue

**5.3.1.3. Western Blotting**

Lysis Buffer (Protein Extraction): 2M NaOH
5% Beta- Mercaptoethanol
50% Trichlor- Acetic Acid

SDS-Running Buffer: (1l) 10x: 144g Glycin
30g Tris Base
10g SDS

10x TBST (1l): 12g Tris
88g NaCl
5g Tween 20 (5ml)

SDS sample buffer:

| | |
|---|---|
| 0,5M Tris-HCl pH 6,8 | 5ml |
| Glycerol | 2,5ml |
| 10% SDS | 4ml |
| Beta-mercaptoethanol | 2ml |
| 0,05% bromophenol blue | 0,2ml |
| dd $H_2O$ | 9,5ml |

4x Separation gel buffer: 1,5MTris-HCl pH8.8

4x Stacking gel buffer: 0,5MTris-HCl pH6.8

20% APS: 1g Ammoniumpersulfat in 5ml $H_2O$ stored at -20°C

Anode1 buffer: 0,3M Tris
20% Methanol
pH 10,4
Anode2 buffer: 2,5mM Tris
20% Methanol
Cathode buffer: 0,04M amino-caprionic acid
20% Methanol
0,01% SDS
Block solution: 25% milk powder in 1x TBST

#### 5.3.1.4. High Efficiency Yeast Transformation

1M Lithium-Acetate:
50% 3350 Polyethylenglycole:
ssCarrier DNA: 10mg/ml

### 5.3.2. Media:

YPD: 1% Yeast Extract
2% Meat- peptone
for plates: 2% Agar
after autoclavation: 2% Glucose; for YPR: 2% Raffinose, for YPGal: 2% Galactose

# 6. Appendix

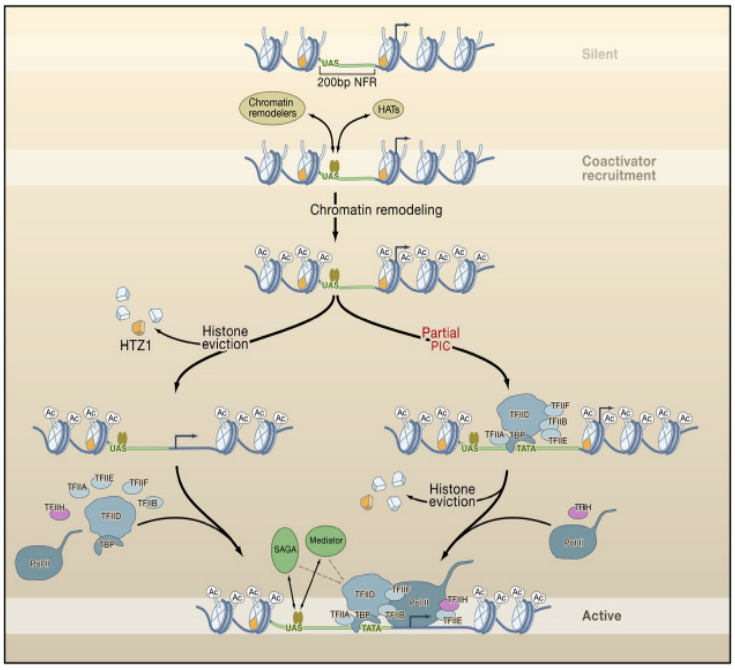



**Figure A1** (Taken from Li et al. Cell 128, 707–719, 2007) Two different models are suggested for the sequence of histone eviction and association of the preinitiation complex (PIC). One model proposes that Htz1 containing nucleosomes are directly lost after acetylation and chromatin remodeling following association of the preinitiation complex (PIC) containing PolII, TFII2D, TFII2A and TFII2B and TFIIH, the other model proposes that partial PIC is bound and Htz1 is lost after association of PolII and TFIIH

| Modifications | Position | | Enzymes | | | | Recognition Module(s)[a] | Functions in Transcription |
|---|---|---|---|---|---|---|---|---|
| | | | *S. cerevisiae* | *S. pombe* | *Drosophila* | Mammals | | |
| Methylation | H3 | K4 | Set1 | Set1 | Trx, Ash1 | MLL, ALL-1, Set9/7, ALR-1/2, ALR, Set1 | PHD, Chromo, WD-40 | Activation |
| | | K9 | n/a | Clr4 | Su(var)3-9, Ash1 | Suv39h, G9a, Eu-HMTase I, ESET, SETBD1 | Chromo (HP1) | Repression, activation |
| | | K27 | | | | E(Z) | Ezh2, G9a | Repression |
| | | K36 | Set2 | | | HYPB, Smyd2, NSD1 | Chromo(Eaf3), JMJD | Recruiting the Rpd3S to repress internal initiation |
| | | K79 | Dot1 | | | Dot1L | Tudor | Activation |
| | H4 | K20 | | Set9 | PR-Set7, Ash1 | PR-Set7, SET8 | Tudor | Silencing |
| Arg Methylation | H3 | R2 | | | | CARM1 | | Activation |
| | | R17 | | | | CARM1 | | Activation |
| | | R26 | | | | CARM1 | | Activation |
| | H4 | R3 | | | | PRMT1 | (p300) | Activation |
| Phosphorylation | H3 | S10 | Snf1 | | | | (Gcn5) | Activation |
| Ubiquitination | H2B | K120/123 | Rad6, Bre1 | Rad6 | | UbcH6, RNF20/40 | (COMPASS) | Activation |
| | H2A | K119 | | | | hPRC1L | | Repression |
| Acetylation | H3 | K56 | | | | | (Swi/Snf) | Activation |
| | H4 | K16 | Sas2, NuA4 | | dMOF | hMOF | Bromodomain | Activation |
| | Htz1 | K14 | NuA4, SAGA | | | | | Activation |

[a] The proteins that are indicated within the parentheses are shown to recognize the corresponding modifications but specific domains have yet to be determined.

**Table A1** (from Li et al. Cell 128, 707–719, 2007) Description of conserved histone modifications in S. cerevisiae, S. pombe. D. melanogaster and Mammals

# 7. Abbreviations

| | |
|---|---|
| Arp | Actin related protein |
| Asf1 | Anti-silencing factor 1 |
| ATP | Adenosin- triphosphate |
| BSA | Bovine serum albumine |
| ChIP | Chromatin Immuno Precipitation |
| CI | Chloroform Isoamylalcohol |
| CTD | C- terminal domain |
| DEPC | Diethylpyrocarbonate |
| DSB | Double strand break |
| FACT | Facilitates Transcription |
| HAT | Histone Acetyltransferase |
| HDAC | Histone Deacteylase |
| Htz1 | Histone variant |
| Ies | Ino eighty subunit |
| ORF | Open reading frame |
| PBS | Phosphate buffered saline |
| PCI | Phenol- chloroform - isoamylalcohol |
| PCR | Polymerase chain reaction |
| PIC | Preinitiation complex |
| Pr | Promoter |
| Rpm | Rounds per minute |
| RT | Room temperature |
| SSC | Sodium chloride/ Sodium citrate |
| TAE | Tris- acetate EDTA |
| TBE | Tris- buffered EDTA |
| TBP | TATA- binding protein |
| TBS | Tris- buffered saline |
| TBST | Tris- buffered saline Tween 20 |
| TCA | Trichlor acetic acid |
| TE | Tris- EDTA |
| UAS | Upstream activating sequence |
| WT | Wild type |

# 8. References


Adkins MW, Tyler JK. (2004) The histone chaperone Asf1p mediates global chromatin disassembly in vivo. *J Biol Chem. Dec 10; 279(50):52069-74.*

Adkins MW, Tyler JK. (2006) Transcriptional activators are dispensable for transcription in the absence of Spt6-mediated chromatin reassembly of promoter regions. *Mol Cell. Feb 3;21(3):405-16.*

Alepuz, P. M., Jovanovic, A., Reiser, V., and Ammerer, G. (2001). Stress-induced map kinase Hog1 is part of transcription activation complexes. *Mol Cell 7, 767-777.*

Allard S, Utley RT, Savard J, Clarke A, Grant P, Brandl CJ, Pillus L, Workman JL, Côté J.NuA4 (1999), an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p.*EMBO J. 15;18(18):5108-19*

Angus-Hill, M. L., Schlichter, A., Roberts, D., Erdjument-Bromage, H., Tempst, P., and Cairns, B. R. (2001). A Rsc3/Rsc30 zinc cluster dimer reveals novel roles for the chromatin remodeler RSC in gene expression and cell cycle control. *Mol Cell 7, 741-751.*

Barbaric, S., Luckenbach, T., Schmid, A., Blaschke, D., Horz, W., and Korber, P. (2007). Redundancy of chromatin remodeling pathways for the induction of the yeast *PHO5* promoter in vivo. *J Biol Chem.*

Basehoar, A. D., Zanton, S. J., and Pugh, B. F. (2004). Identification and distinct regulation of yeast TATA box-containing genes. *Cell 116, 699-709.*

Belotserkovskaya, R., Oh, S., Bondarenko, V. A., Orphanides, G., Studitsky, V. M., and Reinberg, D. (2003). FACT facilitates transcription-dependent nucleosome alteration. *Science 301, 1090-1093.*

Becker PB. (2002) Nucleosome sliding: facts and fiction.*EMBO J. Sep 16;21(18):4749-53. Review*

Bhaumik SR, Green MR. (2002) Differential requirement of SAGA components for recruitment of TATA-box-binding protein to promoters in vivo. *Mol Cell Biol. Nov;22(21):7365-71*

Brachmann, C. B., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P., and Boeke, J. D. (1998). Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast 14, 115-132.*

Cai, Y., Jin, J., Yao, T., Gottschalk, A. J., Swanson, S. K., Wu, S., Shi, Y., Washburn, M. P., Florens, L., Conaway, R. C., and Conaway, J. W. (2007). YY1 functions with INO80 to activate transcription. *Nat Struct Mol Biol.*

Cairns BR, Kim YJ, Sayre MH, Laurent BC, Kornberg RD. (1994) A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. *Proc Natl Acad Sci U S A. Mar 1;91(5):1950-4.*

Cairns BR, Erdjument-Bromage H, Tempst P, Winston F, Kornberg RD. (1998) Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and Swi/Snf.*Mol Cell. Nov;2(5):639-51*

Carey, M., Li, B., and Workman, J. L. (2006). RSC exploits histone acetylation to abrogate the nucleosomal block to RNA polymerase II elongation. *Mol Cell 24, 481-487.*

Causton, H. C., Ren, B., Koh, S. S., Harbison, C. T., Kanin, E., Jennings, E. G., Lee, T. I., True, H. L., Lander, E. S., and Young, R. A. (2001). Remodeling of yeast genome expression in response to environmental changes. *Mol Biol Cell 12, 323-337.*

Chakravarthy S, Bao Y, Roberts VA, Tremethick D, Luger K. (2004) Structural characterization of histone H2A variants.*Cold Spring Harb Symp Quant Biol.;69:227-34. Review*

Downs, J. A., Allard, S., Jobin-Robitaille, O., Javaheri, A., Auger, A., Bouchard, N., Kron, S. J., Jackson, S. P., and Cote, J. (2004). Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Mol Cell 16, 979-990*

Doyon Y, Côté J. (2004)The highly conserved and multifunctional NuA4 HAT complex.*Curr Opin Genet Dev. Apr;14(2):147-54. Review*

Ebbert, R., Birkmann, A., and Schüller, H. J. (1999). The product of the SNF2/SWI2 paralogue *INO80* of *Saccharomyces cerevisiae* required for efficient expression of various yeast structural genes is part of a high-molecular-weight protein complex. *Mol Microbiol 32, 741-751.*

Eisen JA, Sweder KS, Hanawalt PC. (1995) Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res. Jul 25;23(14):2715-23. Review.*

Emerson BM. (2002) Specificity of gene regulation. *Cell. May 3;109(3):267-70. Review*

Fazzio TG, Kooperberg C, Goldmark JP, Neal C, Basom R, Delrow J, Tsukiyama T. (2001) Widespread collaboration of Isw2 and Sin3-Rpd3 chromatin remodeling complexes in transcriptional repression. *Mol Cell Biol. Oct;21 (19):6450-60*

Fazzio TG, Tsukiyama T. (2003) Chromatin remodeling in vivo: evidence for a nucleosome sliding mechanism. *Mol Cell. Nov;12(5):1333-40.*

Fillingham, J., Keogh, M. C., and Krogan, N. J. (2006). GammaH2AX and its role in DNA double-strand break repair. *Biochem Cell Biol 84, 568-577.*

Flaus, A., and Owen-Hughes, T. (2004). Mechanisms for ATP-dependent chromatin remodeling: farewell to the tuna-can octamer? *Curr Opin Genet Dev 14, 165-173.*

Galarneau L, Nourani A, Boudreault AA, Zhang Y, Héliot L, Allard S, Savard J, Lane WS, Stillman DJ, Côté J. (2000) Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. Mol Cell. Jun;5(6):927-37

Gasch, A. P., Spellman, P. T., Kao, C. M., Carmel-Harel, O., Eisen, M. B., Storz, G., Botstein, D., and Brown, P. O. (2000). Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell 11, 4241-4257.*

Goldmark JP, Fazzio TG, Estep PW, Church GM, Tsukiyama T. (2000) The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. *Cell. Oct 27;103(3):423-33*

Görzer, I., Schüller, C., Heidenreich, E., Krupanska, L., Kuchler, K., and Wintersberger, U. (2003). The nuclear actin-related protein Act3p/Arp4p of *Saccharomyces cerevisiae* is involved in transcription regulation of stress genes. *Mol Microbiol 50, 1155-1171.*

Guillemette B, Bataille AR, Gévry N, Adam M, Blanchette M, Robert F, Gaudreau L. (2005) Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. *PLoS Biol. Dec;3(12):e384.*

Huisinga, K. L., and Pugh, B. F. (2004). A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in *Saccharomyces cerevisiae*. *Mol Cell 13, 573-585.*

Izban, M. G., and Luse, D. S. (1991). Transcription on nucleosomal templates by RNA polymerase II in vitro: inhibition of elongation with enhancement of sequence-specific pausing. *Genes Dev 5, 683-696.*

Jacob, F., and Monod, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. *J Mol Biol 3, 318-356.*

Jenuwein, T., and Allis, C. D. (2001). Translating the histone code. *Science 293, 1074-1080.*

Jin, J., Cai, Y., Yao, T., Gottschalk, A. J., Florens, L., Swanson, S. K., Gutierrez, J. L., Coleman, M. K., Workman, J. L., Mushegian, A.*, et al.* (2005). A mammalian chromatin remodeling complex with similarities to the yeast INO80 complex. *J Biol Chem 280, 41207-41212.*

Z.O. Jonsson, S. Jha, J.A.Wohlschlegel, A. Dutta, 2004 Rvb1p/Rvb2p recruit Arp5p and assemble a functional Ino80 chromatin remodeling complex, Mol. Cell 16 465–477.

Joshi and Struhl, (2005) Eaf3 chromodomain interaction with methylated H3-K36 links histone deacetylation to Pol II elongation. *Mol. Cell ;20(6):971-8*

Kaplan, C. D., Laprade, L., and Winston, F. (2003). Transcription elongation factors repress transcription initiation from cryptic sites. *Science 301, 1096-1099.*

Kassabov SR, Zhang B, Persinger J, Bartholomew B. (2003) Swi/Snf unwraps, slides, and rewraps the nucleosome *Mol Cell. 11(2):391-403.*

Katsani KR, Mahmoudi T, Verrijzer CP. (2003) Selective gene regulation by Swi/Snf-related chromatin remodeling factors.*Curr Top Microbiol Immunol.;274:113-41. Review*

Klymenko, T., Papp, B., Fischle, W., Kocher, T., Schelder, M., Fritsch, C., Wild, B., Wilm, M., and Muller, J. (2006). A Polycomb group protein complex with sequence-specific DNA-binding and selective methyl-lysine-binding activities. *Genes Dev 20, 1110-1122.*

Kobor, M. S., Venkatasubrahmanyam, S., Meneghini, M. D., Gin, J. W., Jennings, J. L., Link, A. J., Madhani, H. D., and Rine, J. (2004). A Protein Complex Containing the Conserved Swi2/Snf2-Related ATPase Swr1p Deposits Histone Variant H2A.Z into Euchromatin*. PLoS Biol 2, E131*.

Kristjuhan, A., and Svejstrup, J. Q. (2004). Evidence for distinct mechanisms facilitating transcript elongation through chromatin in vivo. *Embo J 23, 4243-4252.*

Krogan, N. J., Keogh, M. C., Datta, N., Sawa, C., Ryan, O. W., Ding, H., Haw, R. A., Pootoolal, J., Tong, A., Canadien, V.*, et al.* (2003a). A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Mol Cell 12, 1565-1576.*

Krogan, N. J., Kim, M., Tong, A., Golshani, A., Cagney, G., Canadien, V., Richards, D. P., Beattie, B. K., Emili, A., Boone, C.*, et al.* (2003b). Methylation of histone H3 by Set2 in *Saccharomyces cerevisiae* is linked to transcriptional elongation by RNA polymerase II. *Mol Cell Biol 23, 4207-4218.*

Kuras, L., and Struhl, K. (1999). Binding of TBP to promoters in vivo is stimulated by activators and requires Pol II holoenzyme. *Nature 399, 609-613.*

Lee, C. K., Shibata, Y., Rao, B., Strahl, B. D., and Lieb, J. D. (2004). Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat Genet 36, 900-905.*

Lewis, M. (2005). The lac repressor. *C R Biol 328, 521-548.*

Li, B., Carey, M., and Workman, J. L. (2007a). The role of chromatin during transcription. *Cell 128, 707-719.*

Li, B., Gogol, M., Carey, M., Pattenden, S. G., Seidel, C., and Workman, J. L. (2007b). Infrequently transcribed long genes depend on the Set2/Rpd3S pathway for accurate transcription. *Genes Dev 21, 1422-1430.*

Li, B., Howe, L., Anderson, S., Yates, J. R., 3rd, and Workman, J. L. (2003). The Set2 histone methyltransferase functions through the phosphorylated carboxyl-terminal domain of RNA polymerase II. *J Biol Chem 278, 8897-8903.*

Lindstrom, K. C., Vary, J. C., Jr., Parthun, M. R., Delrow, J., and Tsukiyama, T. (2006). Isw1 functions in parallel with the NuA4 and Swr1 complexes in stress-induced gene repression. *Mol Cell Biol 26, 6117-6129.*

Longtine, M. S., McKenzie, A., 3rd, Demarini, D. J., Shah, N. G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J. R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast 14, 953-961.*

Lorch, Y., Maier-Davis, B., and Kornberg, R. D. (2006). Chromatin remodeling by nucleosome disassembly in vitro. *Proc Natl Acad Sci U S A 103, 3090-3093.*

Mason, P. B., and Struhl, K. (2003). The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. *Mol Cell Biol 23, 8323-8333.*

Mellor, J. (2006). Dynamic nucleosomes and gene transcription. *Trends Genet 22, 320-329.*

Mizuguchi, G., Shen, X., Landry, J., Wu, W. H., Sen, S., and Wu, C. (2004). ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science 303, 343-348.*

Mohrmann, L., and Verrijzer, C. P. (2005). Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. *Biochim Biophys Acta 1681, 59-73.*

Morrison, A. J., Highland, J., Krogan, N. J., Arbel-Eden, A., Greenblatt, J. F., Haber, J. E., and Shen, X. (2004). INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. *Cell 119, 767-775.*

Morrison, A. J., Kim, J. A., Person, M. D., Highland, J., Xiao, J., Wehr, T. S., Hensley, S., Bao, Y., Shen, J., Collins, S. R.*, et al.* (2007). Mec1/Tel1 Phosphorylation of the INO80 Chromatin Remodeling Complex Influences DNA Damage Checkpoint Responses. *Cell 130, 499-511.*

Narlikar GJ, Fan HY, Kingston RE. (2002) Cooperation between complexes that regulate chromatin structure and transcription.*Cell. Feb 22;108(4):475-87. Review*

Ng, H. H., Robert, F., Young, R. A., and Struhl, K. (2003). Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol Cell 11, 709-719.*

Nonet, M., Scafe, C., Sexton, J., and Young, R. (1987). Eucaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. *Mol Cell Biol 7, 1602-1611.*

Ohkuni, K., Shirahige, K., and Kikuchi, A. (2003). Genome-wide expression analysis of NAP1 in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun 306, 5-9.*

Orphanides, G., LeRoy, G., Chang, C. H., Luse, D. S., and Reinberg, D. (1998). FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell 92, 105-116.*

Orphanides, G., and Reinberg, D. (2002). A unified theory of gene expression. *Cell 108, 439-451.*

Orphanides, G., Wu, W. H., Lane, W. S., Hampsey, M., and Reinberg, D. (1999). The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. *Nature 400, 284-288.*

Papamichos-Chronakis, M., Krebs, J. E., and Peterson, C. L. (2006). Interplay between Ino80 and Swr1 chromatin remodeling enzymes regulates cell cycle checkpoint adaptation in response to DNA damage. *Genes Dev 20, 2437-2449.*

Pokholok, D. K., Harbison, C. T., Levine, S., Cole, M., Hannett, N. M., Lee, T. I., Bell, G. W., Walker, K., Rolfe, P. A., Herbolsheimer, E.*, et al.* (2005). Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell 122, 517-527.*

Pray-Grant, M. G., Daniel, J. A., Schieltz, D., Yates, J. R., 3rd, and Grant, P. A. (2005). Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature 433, 434-438.*

Reinberg, D., and Sims, R. J., 3rd (2006). de FACTo nucleosome dynamics. *J Biol Chem 281, 23297-23301.*

Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., and Bonner, W. M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem 273, 5858-5868.*

Rosonina and Manley, (2005) From transcription to mRNA: PAF provides a new path *Mol Cell. Oct 28;20(2):167-8.*

Schwabish, M. A., and Struhl, K. (2004). Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. *Mol Cell Biol 24, 10111-10117.*

Schwabish, M. A., and Struhl, K. (2006). Asf1 mediates histone eviction and deposition during elongation by RNA polymerase II. *Mol Cell 22, 415-422.*

Schwabish, M. A., and Struhl, K. (2007). The Swi/Snf Complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation in vivo. Mol Cell Biol.

Shahbazian MD, Zhang K, Grunstein M. (2005) Histone H2B ubiquitylation controls processive methylation but not monomethylation by Dot1 and Set1. *Mol Cell. Jul 22;19(2):271-7*

Shen, X., Mizuguchi, G., Hamiche, A., and Wu, C. (2000). A chromatin remodeling complex involved in transcription and DNA processing. *Nature 406, 541-544.*

Shen, X., Ranallo, R., Choi, E., and Wu, C. (2003a). Involvement of actin-related proteins in ATP-dependent chromatin remodeling. *Mol Cell 12, 147-155.*

Shen, X., Xiao, H., Ranallo, R., Wu, W. H., and Wu, C. (2003b). Modulation of ATP-dependent chromatin-remodeling complexes by inositol polyphosphates. *Science 299, 112-114.*

Simic, R., Lindstrom, D. L., Tran, H. G., Roinick, K. L., Costa, P. J., Johnson, A. D., Hartzog, G. A., and Arndt, K. M. (2003). Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. *Embo J 22, 1846-1856.*

Sudarsanam, P., Iyer, V. R., Brown, P. O., and Winston, F. (2000). Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A 97, 3364-3369.*

Tsukuda, T., Fleming, A. B., Nickoloff, J. A., and Osley, M. A. (2005). Chromatin remodeling at a DNA double-strand break site in *Saccharomyces cerevisiae*. *Nature 438, 379-383.*

Tsukiyama T, Palmer J, Landel CC, Shiloach J, Wu C. (1999) Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in Saccharomyces cerevisiae. *Genes Dev. Mar 15;13(6):686-97*

van Attikum, H., Fritsch, O., and Gasser, S. M. (2007). Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. *Embo J 26, 4113-4125.*

van Attikum, H., Fritsch, O., Hohn, B., and Gasser, S. M. (2004). Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. *Cell 119, 777-788.*

van Vugt, J. J., Ranes, M., Campsteijn, C., and Logie, C. (2007). The ins and outs of ATP-dependent chromatin remodeling in budding yeast: biophysical and proteomic perspectives. *Biochim Biophys Acta 1769, 153-171.*

Wang W. (2003) The Swi/Snf family of ATP-dependent chromatin remodelers: similar mechanisms for diverse functions. *Curr Top Microbiol Immunol. 274:143-69. Review.*

Weber, V., Harata, M., Hauser, H., and Wintersberger, U. (1995). The actin-related protein Act3p of *Saccharomyces cerevisiae* is located in the nucleus. *Mol Biol Cell 6, 1263-1270.*

Workman, J. L., and Kingston, R. E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu Rev Biochem 67, 545-579.*

Wong MC, Scott-Drew SR, Hayes MJ, Howard PJ, Murray JA. (2002) RSC2, encoding a component of the RSC nucleosome remodeling complex, is essential for 2 microm plasmid maintenance in Saccharomyces cerevisiae. *Mol Cell Biol. Jun;22(12):4218-29*

Zabaronick, S. R., and Tyler, J. K. (2005). The histone chaperone anti-silencing function 1 is a global regulator of transcription independent of passage through S phase. *Mol Cell Biol 25, 652-660.*

Zhang, H., Roberts, D. N., and Cairns, B. R. (2005a). Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell 123, 219-231.*

Zhang, L., Schroeder, S., Fong, N., and Bentley, D. L. (2005b). Altered nucleosome occupancy and histone H3K4 methylation in response to 'transcriptional stress'. *Embo J 24, 2379-2390.*

Zhang, Y., Smith, C. L., Saha, A., Grill, S. W., Mihardja, S., Smith, S. B., Cairns, B. R., Peterson, C. L., and Bustamante, C. (2006). DNA translocation and loop formation mechanism of chromatin remodeling by Swi/Snf and RSC. *Mol Cell 24, 559-568.*

Zhao, J., Herrera-Diaz, J., and Gross, D. S. (2005). Domain-wide displacement of histones by activated heat shock factor occurs independently of Swi/Snf and is not correlated with RNA polymerase II density. *Mol Cell Biol 25, 8985-8999.*

Zofall, M., Persinger, J., Kassabov, S. R., and Bartholomew, B. (2006). Chromatin remodeling by ISW2 and Swi/Snf requires DNA translocation inside the nucleosome*. Nat Struct Mol Biol 13, 339-346.*

# 9. Curriculum Vitae

**Persönliche Daten:**

| | |
|---|---|
| Name: | Eva Klopf |
| Geboren am: | 15.1.1981 |
| Staatsbürgerschaft: | Österreich |
| Anschrift: | Sickenberggasse 6/4 |
| | A-1190 Wien |
| Telefonnummer | 0699/11453954 |
| E-Mail-Adresse | eva.klopf@univie.ac.at |

**Ausbildung:**

| | |
|---|---|
| 1987 – 1991 | Volksschule Zennerstrasse 1140 Wien |
| 1991 – 1999 | AHS BRG14 Linzerstrasse, Matura 1999 mit Auszeichnung |
| Okt. 1999- Sept. 2000 | Studium der Chemie an der Universität Wien |
| Seit Okt. 2000 | Studium der Molekularen Biologie an der Universität Wien |
| Seit Juni 2006 | Diplomarbeit bei Dr. Christoph Schüller, |
| | Max F. Perutz Laboratories, Dr. Bohrgasse 9 |
| | 1190 Wien |