universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

„Interplay of Chromatin Remodeling and Stress Gene Repression: Recruitment and Functions of the INO80 Complex“

Verfasser

Gerhard Niederacher

angestrebter akademischer Grad

Magister der Naturwissenschaften (Mag.rer.nat.)

Wien, 2011

Studienkennzahl lt. Studienblatt:	A490
Studienrichtung lt. Studienblatt:	Molekulare Biologie
Betreuer:	Dr. Christoph Schüller

Danksagung

Ich möchte mich bei Christoph Schüller und Eva Klopf für die angenehme und sehr lehrreiche Zusammenarbeit bedanken. Beide haben mich während meiner Diplomarbeit hervorragend betreut und es immer wieder geschafft mich zu motivieren. Ich bedanke mich auch bei allen Mitgliedern der Ammerer-, Schüller- und ehemaligen Kragler-Gruppe für die äußerst kollegiale Arbeitsatmosphäre. Insbesondere Daniela Fichtenbauer, Gregor Kollwig, Wolfgang Reiter und Jiři Veis standen mir bei Problemen jederzeit mit Rat und Tat zur Seite.

Ganz besonders möchte ich mich bei meinen Eltern Erna und Fritz Niederacher sowie bei meinen Großeltern bedanken, die mich während der gesamten Studienzeit unterstützt haben.

CONTENTS

The Introduction part of this diploma thesis represents an adapted and updated version of a Review article entitled “Interplay of dynamic transcription and chromatin remodeling: lessons from yeast” published in the International Journal of Molecular Sciences (Niederacher et al. 2011)

Niederacher, G., E. Klopf and C. Schüller (2011). "Interplay of dynamic transcription and chromatin remodeling: lessons from yeast." International journal of molecular sciences **12**(8): 4758-4769.

I contributed to the concept and provided the majority of the text and one figure. Eva Klopf helped to finalize the text and provided one figure. Christoph Schüller contributed to the concept and revision of this manuscript.

1. Introduction

Eukaryotic DNA is wrapped around histone octamers, which are composed of dimers of the histones H2A, H2B, H3 and H4. 147bp of DNA are wrapped 1,65 times around each octamer forming nucleosomes, the basic packaging units of chromatin (Luger et al. 1997). Nucleosomes, connected by linker DNA of variable length as “beads on a string”, generate a 11nm linear structure. The linker histone H1 is positioned at the top of the histone octamer and enables higher organized compaction of DNA into transcriptionally inactive 30nm fibres. In addition to topological DNA compaction chromatin structure exhibits an important regulatory role on several cellular processes including transcription, replication, silencing and repair of DNA damage. To understand the role of chromatin for regulation of transcription it is important to know where nucleosomes are positioned and how positioning is achieved. Genome wide mappings of nucleosomes in *S.cerevisiae* revealed that many genes show highly positioned nucleosomes flanking a nucleosome depleted region (NDR) upstream of transcriptional start sites and downstream of stop codons (Yuan et al. 2005). These positioned nucleosomes are usually referred to as +1 and -1 for the nucleosome near the transcriptional start site and the first 5´ nucleosome, respectively. In contrast, nucleosomes within the open reading frame of coding genes are less strictly positioned (reviewed in (Jiang et al. 2009)). Here we discuss how the reorganization of chromatin structure contributes to adaptation of transcriptional programs for particular situations and requirements.

Basically there are three groups of activities which change chromatin structure during transcription. Histone modifiers introduce posttranslational, covalent modifications to histone tails and thereby change the contact between DNA and histones. These modifications govern access of regulatory factors. Histone chaperones aid eviction and positioning of histones. A third class of chromatin reconstructing factors are ATP dependent chromatin remodelers. These multi-subunit complexes utilize energy from ATP hydrolysis for various chromatin remodeling activities including nucleosome sliding, nucleosome displacement and the incorporation and exchange of histone variants.

Living cells need to adjust gene transcription according to diverse internal and external parameters. These signals are transmitted to the nucleus by various pathways where they trigger changes in gene expression. In principle, transcription of RNA polymerase II (RNA Pol II) dependent genes can be categorized in three different

patterns according to the type of inducing signals (reviewed in (Yosef et al. 2011)). As shown in figure 1, these are sustained transcription, single pulses and oscillations.

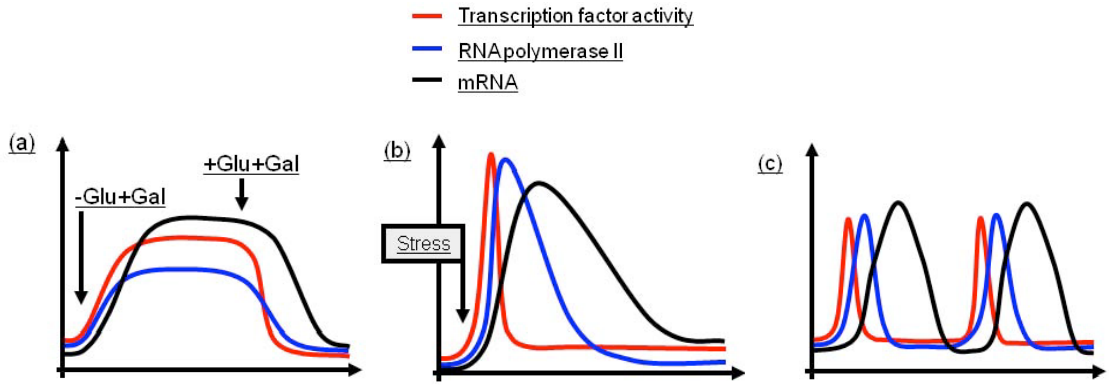


Figure 1. Patterns of induced transcription. **(a)** Sustained transcription is characterized by prolonged transcription factor activity depending on the induction signal resulting in moderate RNA Pol II association and sustained transcript levels. **(b)** During single pulse transcription intense transcription factor activity is followed by high RNA Pol II occupancy over a short period of time resulting in swift upregulation of transcripts and subsequent repression to basal levels. **(c)** Oscillatory transcription appears as a circular pattern characterized by short and strong transcription factor activity as well as RNA Pol II binding. Transcripts are quickly and strongly upregulated following a harsh repression to basal levels.

Induced sustained transcription patterns switch expression of repressed genes more or less rapidly to an induced state and occur frequently during changes of metabolic programs (Fig.1a). A classic example is the regulation of the yeast *GAL1/10* locus encoding products required for galactose metabolism. *GAL1* transcription is upregulated and sustained as long as galactose is available and glucose is absent. In contrast, induced single pulse responses occur when cells encounter environmental stress such as high external osmolarity or heat shock. In these situations, transcripts are rapidly induced followed by adaptation and reduction to basal levels (Fig.1b) (Chechik et al. 2009). Finally, oscillatory expression patterns are characterized by periodic transcription and can be found in genes regulated by cell cycle and circadian rhythm (Fig.1c). In this work I focus primarily on the role of chromatin for transcriptional regulation of stress genes. Stress induced genes have a characteristic transiently, pulsed expression pattern. In the following I will give a short introduction into the current knowledge how chromatin remodeling interplays with transcriptional regulation, exemplified by intensively studied loci in yeast.

1.1 Regulation of transcriptional activation

Induction of gene transcription is triggered by the binding of transcriptional activators to specific promoter elements (upstream activation sequences) followed by recruitment of co- activators such as Mediator and chromatin reconstructing factors (e.g. SAGA, SWI/SNF, RSC). Thereafter, components of the general transcription machinery including TATA binding protein (TBP), general transcription factors (GTFs) and RNA Pol II assemble into the pre- initiation complex (PIC). Some loci contain preformed PICs which are paused for transcription and require additional factors for release into productive elongation (Rougvie et al. 1988). However, in yeast the rate limiting step of induced transcription is activator dependent formation of the PIC. Histones are displaced in front of elongating RNA Pol II and rapidly reconstituted behind. The force of transcribing RNA Pol II could be an important factor for histone displacement. Some chromatin reconstructing factors are recruited to the coding regions of transcribed genes where they assist RNA Pol II during elongation. A prominent example is the histone chaperone Asf1 which co- migrates with RNA Pol II and facilitates H3-H4 eviction (Schwabish et al. 2006).

The physicochemical properties of DNA are almost uniform. Hence, DNA-sequence dependent processes such as transcription factor binding need to be localized on the long molecule. Chromatin structure is important for marking promoters and transcription units much like the flag on the golf green. One important feature of chromatin is the definition of the nucleosome depleted region (NDR) immediately flanked by positioned nucleosomes at promoter regions (Jiang and Pugh 2009; Bai et al. 2011). During activation of transcription, nucleosomes are frequently depleted from the promoter by certain chromatin remodeling factors (Fig. 2) such as the SWI/SNF, RSC, SWR1, INO80. These are related but have defined activities, which are partially overlapping.

One further characteristic feature of promoter chromatin is the presence of histone variants. The ATP dependent chromatin remodeling activity of the SWR1 complex is responsible for the exchange of canonical H2A-H2B dimers by H2A-H2A.Z of the +1 nucleosome (Mizuguchi et al. 2004). The reverse reaction removes H2A.Z from chromatin and was recently identified to be INO80 dependent (Papamichos-Chronakis et al. 2011). Two thirds of all nucleosomes in *S.cerevisiae* contain this histone variant which is encoded by the *HTZ1* gene (Guillemette et al. 2005). Genome wide maps revealed that H2A.Z is globally located at the promoter regions of inactive genes in

euchromatin keeping them in a state which is poised for transcriptional activation (Guillemette et al. 2005; Raisner et al. 2005; Zhang et al. 2005). Thus, H2A.Z was suggested to prevent expansion of silent chromatin into transcriptionally active euchromatin. Recent in vitro data indicate that SWR1 mediated deposition of H2A.Z depends on acetylation of H2A and H4 by NuA4 histone acetyltransferase (HAT) complexes (Altaf et al. 2010). NuA4 contains the Esa1 HAT subunit, shares four subunits with SWR1 and was also identified to acetylate H2A.Z after its deposition into chromatin (Keogh et al. 2006). However, the molecular mechanism by which H2A.Z contributes to regulation of transcription is still unknown. H2A.Z containing nucleosomes could act as signals for guiding activators, co- activators or general transcription factors to their appropriate positions. A recent study reported that H2A.Z also influences transcriptional elongation by promoting efficient chromatin remodeling and by stabilization of RNA Pol II elongation complexes (Santisteban et al. 2011).

The regulation of eukaryotic promoters is a complicated process involving many different activities partly dependent on the regulatory mode as described above. A thoroughly studied example of a regulation by a sustained transcriptional switch is the yeast bidirectional *GAL1/10* promoter targeted by the Gal4 transactivator. *GAL1* encodes a galactokinase required for one of the first steps in galactose metabolism. In absence of galactose, Gal4 is bound by the repressor Gal80 and kept inactive. Galactose promotes binding of the regulator Gal3 to Gal80 and thus enables Gal4 to interact with co- activators (reviewed in (Traven et al. 2006)). Gal4 binding to its upstream activating sequence (UASg) is assisted by the RSC chromatin remodelling complex. RSC, an assembly of 15 subunits, is closely related to the highly conserved SWI/SNF chromatin remodeling complex. Both contain an ATPase subunit comprising a DNA binding bromodomain and share two Actin related proteins: Arp7 and Arp9 (Cairns et al. 1998; Hassan et al. 2002). RSC is located at the UASg where it partially unwinds a single nucleosome to promote Gal4 binding (Floer et al. 2010). One of the first co-activators recruited by Gal4 is the yeast SAGA complex containing 21 conserved subunits including the histone acetyltransferase (HAT) Gcn5 (Larschan et al. 2001). Gcn5 acetylates specific lysine residues located on N-terminal tails of histones H3 and H4 (Kuo et al. 1996). Although SAGA was shown to be essential for PIC formation at the *GAL1/10* promoter, its HAT activity is not. A strain lacking the Gcn5 HAT subunit is still able to form a functional PIC, whereas absence of one conserved subunit (Spt3) of SAGA strongly reduces PIC formation (Bhaumik et al. 2001; Bryant et al. 2003). The

SAGA complex seems to have important structural functions for PIC assembly in addition to its catalytic activity. In addition to RSC and SAGA, SWI/SNF is recruited to the *GAL1/10* promoter in presence of galactose where it is involved in the rapid removal of nucleosomes enabling Gal4 to bind additional sites. Deletion of the SWI/SNF subunit Snf2 reduces nucleosome removal from the promoter. Consequently, induction of transcription is delayed, although the overall *GAL1* transcript levels are not reduced (Bryant et al. 2008). Association of SWI/SNF partially depends on histone modifying complexes. Histone acetylation increases SWI/SNF binding and nucleosome displacement by SAGA and NuA4 complexes (Hassan et al. 2001; Chandy et al. 2006; Bryant et al. 2008). Thus, at the GAL1/10 promoter several activities interplay for fine-tuning of transcriptional regulation.

The yeast PHO genes were used for pioneering studies on the influence of chromatin structures during induced transcription (Almer et al. 1986; Schmid et al. 1992; Korber et al. 2004). The *PHO5* gene encodes an acid phosphatase required for mobilization of phosphate. Phosphate starving conditions cause the activators Pho2 and Pho4 to bind upstream activating sequences UASp1 and UASp2 to induce *PHO5* transcription. For transcriptional activation of this locus SAGA, SWI/SNF and the chromatin remodeling complex INO80 are involved in transcriptional activation (Gregory et al. 1999; Barbaric et al. 2007). Similar to the *GAL* locus, co- activators are not essential for achieving the maximum transcript levels of *PHO5* (Barbaric et al. 2007). In fact, they support promoter opening which is responsible for rapid upregulation of transcription. In contrast, the more weakly induced *PHO8* promoter is to a greater extend dependent on chromatin remodeling factors. *PHO8* activation strictly requires Snf2 and the SAGA associated HAT Gcn5. Both, the *PHO5* and *PHO8* loci require the histone chaperone Asf1 for PIC formation (Adkins et al. 2004; Korber et al. 2006; Adkins et al. 2007).

Interaction between chromatin remodeling complexes has also early been observed at the *INO1* promoter (Ebbert et al. 1999). The *INO1* gene product is involved in phospholipid biosynthesis and its transcription is repressed in presence of inositol and choline (Hirsch et al. 1986). In absence of these metabolites, *INO1* promoter nucleosomes are mobilized by INO80 and SWI/SNF to activate transcription (Ford et al. 2007). Recruitment of the SWI/SNF complex to the *INO1* promoter region depends on the presence of the Ino80 ATPase subunit (Ford et al. 2008). Together this suggests for sustained transcribed genes that weakly regulated promoters are more susceptible to

subtle changes of chromatin and thus to activity of chromatin remodeling factors, compared to strong promoters with a switch-like characteristic.

Stress response genes are generally characterized by rapid and strong upregulation in response to cellular stress such as high osmolarity or heat shock followed by downregulation to almost basal levels. This pattern of transcription is referred to as “single pulse transcription” (Fig.1a). One of the best studied conditions is increased extracellular osmolarity which is sensed and transmitted by the mitogen activated protein (MAP) kinase cascade called hyperosmolarity glycerol response (HOG) pathway. Activation of the Map kinase Hog1 leads to its translocation into the nucleus and subsequent activation of genes via certain transcription factors. The transcriptional factor Smp1 is phosphorylated and activated by Hog1. Hog1 also activates the transcription factor Hot1 by phosphorylation. Importantly, Hot1 dependent genes show interactions of Hog1 with components of the PIC such as Mediator and RNA Pol II and directly associate to chromatin (Alepuz et al. 2001; Alepuz et al. 2003). Hog1 binds to the stress transcription factors Msn2 and Msn4 and becomes recruited to chromatin but does not phosphorylate them. Chromatin remodeling is facilitated by Hog1 induced recruitment of RSC to activated stress genes. Furthermore, Hog1 directs the Rpd3L deacetylase complex to promoters which is required for the induction of environmental stress response genes (De Nadal et al. 2004; Alejandro-Osorio et al. 2009; Ruiz-Roig et al. 2010). The histone deacetylase Rpd3 is a subunit of two complexes: Rpd3L and Rpd3S. While Rpd3L has an activating role for stress gene transcription, the repressive roles which have been associated with both complexes will be discussed later. These observations demonstrate a much more general role of MAP kinases for transcriptional regulation. Interaction between a stress activated protein kinase and transcription factors was also confirmed in higher eukaryots (Ferreiro et al. 2010). At heat shock genes the chromatin remodelers RSC, SWI/SNF and ISWI are involved in transcriptional activation. Preloading of the transcriptional activator HSF requires ISWI and RSC complexes indicating that at heat shock genes, similar to the *GAL1/10* promoter, chromatin remodeling occurs prior to activator binding (Erkina et al. 2010).

Taken together, induced transcription strongly depends on activator dependent recruitment of co- activators to form a functional PIC. These co- activators promote acetylation and deacetylation of histone tails, incorporation of histone variants and ATP dependent chromatin remodeling. In case of the strongly induced *GAL1* and *PHO5* promoters, histone acetylation and nucleosome remodeling are not essential for PIC

formation. These activities have a more important role in facilitating swift upregulation of transcript levels by removal of promoter nucleosomes. During elongation several chromatin reconstructing factors travel with elongating RNA Pol II and assist in removing nucleosomes and restoration of canonical chromatin.

1.2 Repressive role of Chromatin

Gene activation and elongation correlate with intense displacement of nucleosomes at promoter and transcribed regions (Fig.2) (Schwabish and Struhl 2006).

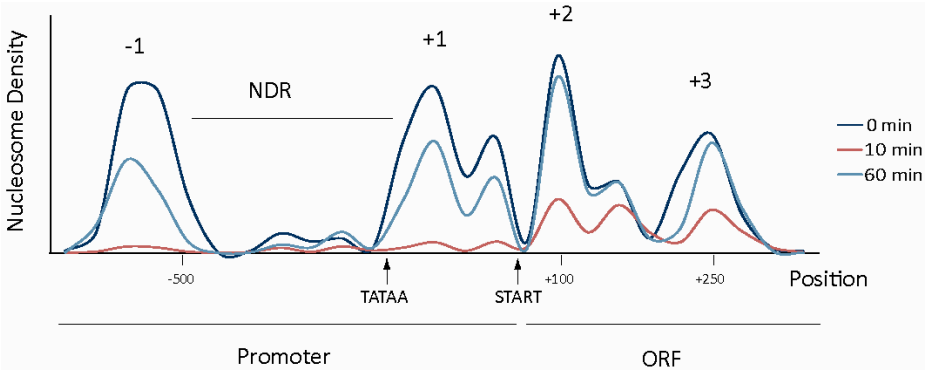


Figure 2. Nucleosome Positions at the stress inducible *CTT1* locus: The schematic representation shows 4 positioned nucleosomes. During uninduced conditions, two (-1 and +1) are flanking the nucleosome depleted region (NDR) and belong to the promoter, whereas nucleosomes +2 and +3 are positioned at the 5' end of the 1,7 kb long ORF (dark blue line). After induction by hyperosmolarity stress for 10min (red line) nucleosome levels are severely depleted in the entire region, after 60 min (light blue line) chromatin structure at the ORF reaches uninduced levels whereas promoter nucleosomes are not completely reassociated. Text and Figure provided by Eva Klopf (unpublished observations).

Disturbed chromatin structures need to be restored to facilitate efficient repression of target genes and to avoid transcription from cryptic initiation sites (Kaplan et al. 2003; Mason et al. 2003; Schwabish and Struhl 2006). The events that promote restoration are promoter closing as well as repositioning of displaced nucleosomes in coding regions. Additionally, deacetylation of histones plays an important role in this respect resulting in tight packaging of chromatin. Similar to activation, transcriptional repression requires several chromatin reconstructing factors such as histone chaperones, chromatin remodelers and histone modifiers.

Interestingly, Msn2 dependent stress genes are upregulated in strains lacking Isw1 in combination with NuA4 or SWR1 (Lindstrom et al. 2006). As already mentioned, the histone deacetylase Rpd3 is a subunit of two complexes: Rpd3L and Rpd3S. Both have been associated with transcriptional repression. The Rpd3L large histone deacetylase complex (HDAC) is recruited to the *INO1* promoter where it removes H4 K5 acetylation under non- induced conditions (Rundlett et al. 1998). Rpd3S represses transcription of target genes and initiation from cryptic sites by deacetylation within coding regions (Carrozza et al. 2005; Keogh et al. 2005). These observations suggest that deacetylation of histones in promoter regions suppresses transcription prior to induction, whereas deacetylation of histones in coding regions supports downregulation and avoids initiation from cryptic sites. At the level of initiation HDACs create compacted, hypoacetylated regions that suppress PIC formation at promoter regions. During elongation these modifiers as well as elongation factors associate to coding regions to avoid cryptic transcription (Kaplan et al. 2003; Mason and Struhl 2003). Histone chaperones also function in the repression of induced genes. They are predominantly associated with elongating RNA Pol II and restore canonical chromatin structure. Asf1 and Spt6 have been intensively studied for their activities during transcriptional regulation. Asf1 interacts with H3-H4 dimers and is associated to promoters and coding regions of active genes. In case of the inducible *GAL1/10* system Asf1 is necessary for histone eviction and positioning and thus is involved in activation and repression (Schwabish and Struhl 2006). In case of some stress induced genes Asf1 has a strictly repressive role and is not required for activation (Klopf et al. 2009). The histone chaperone Spt6 interacts directly with phosphorylated Serine2 of RNA Pol II CTD (Yoh et al. 2007). Spt6 has been described as a repressive factor with a varying role dependent on the particular gene. The mechanism of how Spt6 influences transcription is not known yet. At highly induced genes absence of Spt6 leads to loss of open reading frame nucleosomes and in case of the serine- inducible *CHA1* locus to the delocalization of the +1 nucleosome (Ivanovska et al. 2011). Spt6 is required for repression of strongly induced stress genes (Klopf et al. 2009). In addition to Spt6, the ATP dependent chromatin remodeling complex INO80 is involved in transcriptional repression of stress genes. The INO80 complex contains 15 subunits including the essential subunits Arp4, Actin, Rvb1, Rvb2 and the non-essential Ino80 ATPase, Arp5, Arp8, Nhp10, Taf14 as well as six Ino eighty subunits (Ies1 to 6) (Bao et al. 2007) (Fig.3). Nuclear Actin, Arp4 and Arp8 form a module and interact with the N-terminal

HSA domain of the Ino80 ATPase. Interestingly, all subunits of this module are highly conserved and in absence of Arp8, Actin and Arp4 are not found in the complex (Shen et al. 2000; Shen et al. 2003; Szerlong et al. 2008) (Fig.3). Actin is a component of several chromatin remodeling complexes, however, its precise role in the nucleus is currently unknown. Recent structural data of Arp4 and Arp8 suggest that they prevent nuclear Actin from forming filaments in the nucleus (Fenn et al. 2011). Furthermore Arp4 and Arp8 preferentially interact with ADP- and not ATP- bound Actin. Thus, ATP binding to nuclear Actin could initiate dissociation of the module or the whole complex (Fenn et al. 2011; Kast et al. 2011) (Fig.3).

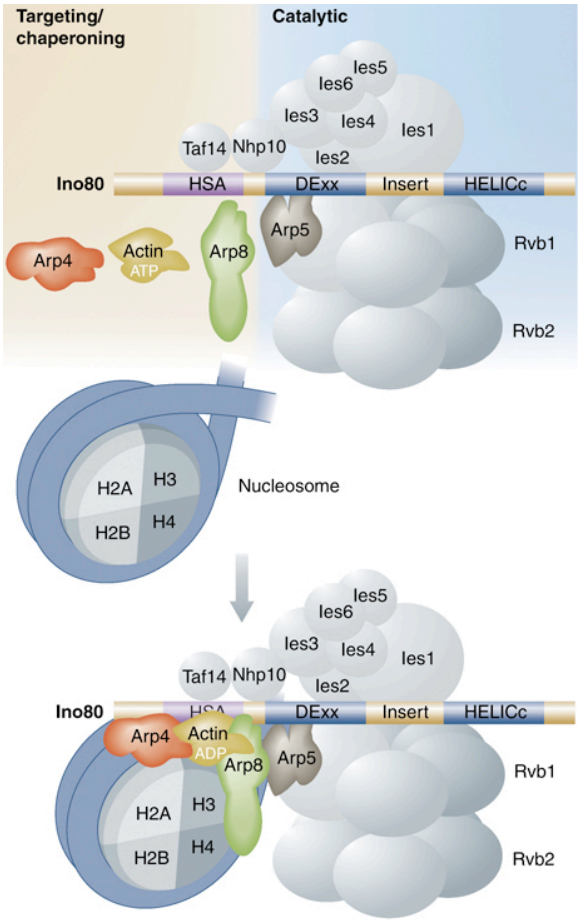


Figure 3. Schematic illustration of the INO80 complex. The N-terminal HSA domain of the Ino80 ATPase is conserved in yeast, human and fly and the binding site for a module of nuclear Actin, Arp4 and Arp8. Recent structural data indicate that GDP binding to Actin increases the affinity to Arp4 and Arp8 and could be the main step for assembly, whereas GTP binding to Actin could be responsible for degradation of the Actin/Arp-module. Figure from (Kast and Dominguez 2011)

The INO80 complex is involved in the regulation of transcription, replication and repair of double strand breaks (Bao et al. 2011). Strains lacking the INO80 subunit Arp4

or Arp8 show increased and prolonged transcription of stress genes (Görzer et al. 2003; Klopf et al. 2009) (Fig.4). This effect was observed for several genes induced by high osmolarity, heat shock or copper stress (Klopf et al. 2009). Recent high-resolution microarray transcript profiles under osmotic stress comparing a wild type to an *arp8Δ* strain indicate that the INO80 complex has a global role for transcriptional repression at highly induced genes (Eva Klopf, unpublished data).

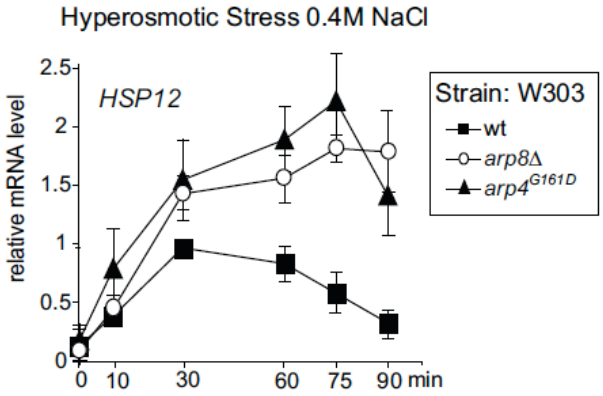


Figure 4. *HSP12* transcript levels (relative to *IPP1*) of *arp8Δ* and *arp4*^{G161D} cells compared to the wild type. Similar to the arp8Δ strain, a strain carrying a temperature sensitive allele of *ARP4* (*arp4*^{G161D}) shows a hyperinduced transcription phenotype at the non-permissive temperature. Figure from (Klopf et al. 2009)

The hyperinduction of transcription in *arp8Δ* mutants coincides with a delay in restoration of chromatin structure along promoter and open reading frames (Klopf et al. 2009). The unique expression kinetics of stress induced genes might cause disturbance of chromatin leading to generation of a signal attracting INO80. Stress genes are easily inducible and characterized by rapid upregulation with transiently high transcript levels, followed by adaptation and repression to basal transcription (Brown et al 2000, see Fig.1b). Strikingly, these genes exhibit reduced nucleosome density during active transcription (Gross et al. 2005). Therefore, stress genes are an advantageous model system for studying the role of INO80 during active transcription. After induction, the INO80 complex is recruited to coding regions where it is involved in repositioning of evicted histones after transcribing RNA Pol II. Although the general function of INO80 at stress genes has been described, the underlying molecular mechanism remains unclear. Chromatin Immunoprecipitation assays indicate that the Ino80 subunit is preferentially recruited to coding regions and not to promoters of induced genes (Klopf

2009). This would suggest that the complex is important for reconstitution of chromatin structure within the open reading frame. However, recent data of Nucleosome Scanning Assays have demonstrated that the promoter nucleosomes (-1 and +1) are strongly affected in absence of the subunit Arp8. In wild type strains, induction of the transcription leads to massive depletion of promoter nucleosomes at the *CTT1* locus after 10min hyperosmolarity stress. Nucleosomes are again repositioned within 60min. In a mutant of the INO80 complex lacking the Arp8 subunit, repositioning of -1 and +1 nucleosomes is significantly delayed (Eva Klopf, unpublished data).

Furthermore, the recruitment mechanism of the complex to distinct loci is unknown. The INO80 complex is supposed to bind to either free DNA, histone modifications or to a specific chromatin associated factor. Histone modifications could act as landmarks guiding the complex to the proper positions. For example, phosphorylation of H2A Serine 129 (γ-H2AX) is required for recruitment of INO80 to double strand breaks (DSB) and this interaction is mediated by Nhp10 (Morrison et al. 2004). The mammalian INO80 complex accumulates at double strand break sites independent of γ-H2AX phosphorylation and Arp8 seems to be important for this process (Kashiwaba et al. 2010). Further, co-transcriptional methylation of histone tails could act as recruitment signals for INO80. Both, γ-H2AX phosphorylation and trimethylation of H3K4 and H3K36 are not required for recruitment of INO80 to transcription sites. A strain carrying a H2A mutant allele which cannot be phosphorylated at S129 (H2A S129A), as well as mutants lacking the histone methylases Set1 and Set2 show a wild type like recruitment kinetic of Ino80 to stress genes (Klopf et al. 2009). In addition to the identification of chromatin landmarks which govern INO80 binding, a second way to approach the recruitment mechanism of the complex is to find the subunits which mediate its interaction with chromatin. Earlier studies indicated that Arp8 is possibly not required for targeting the Ino80 ATPase subunit to stress gene coding regions. This avenue of research was continued here with a more careful approach.

2. Aim of this Work

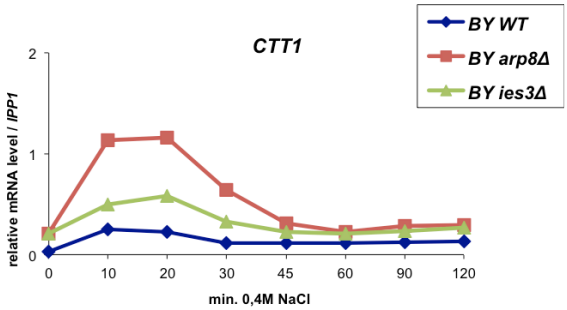
Aim of this work was the identification of INO80 subunits which contribute to transcriptional repression of stress genes. The idea was to determine the specific roles of these key players for either recruitment or function of INO80. Since INO80 was associated with repression of stress genes, these mutants were expected to show hyperinduced transcription resulting in elevated mRNA levels. I screend mutants lacking individual non-essential subunits of the INO80 complex for a hyperinduced transcription phenotype to identify key components of INO80 for the regulation of stress gene transcription. The criteria for being a key player was that the hyperinduced transcription phenotype is similar or even stronger compared to the phenotype observed in the *arp8Δ* mutant. Deletion of a single INO80 subunit could influence the recruitment mechanism as well as the functionality of the complex. Subsequently, I tried to differentiate the functional role of the identified components either for recruitment or for activity of the INO80 complex.

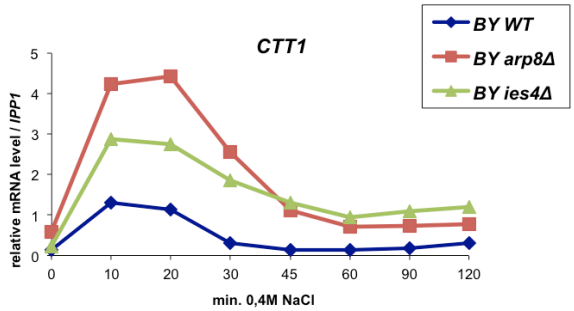
3. Results

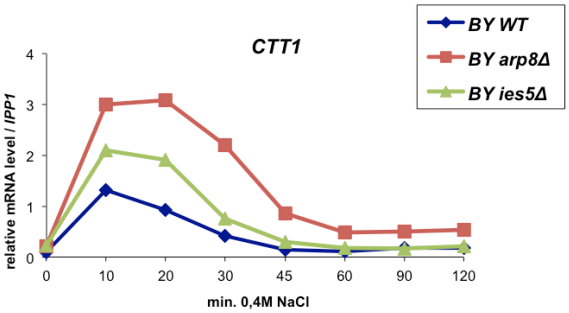
3.1 Actin related Proteins (Arps) are Key Players for INO80 Action at Stress Genes.

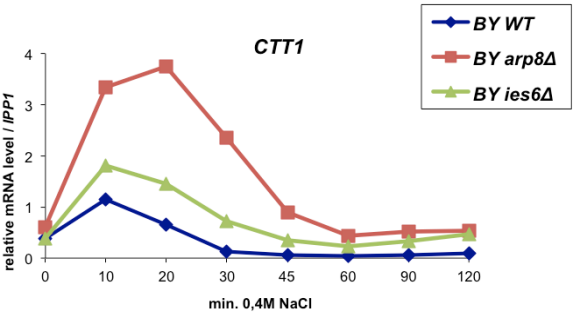
I screened mutants of the non-essential INO80 subunits for a hyperinduced transcription phenotype to identify the relevant subunits for transcriptional repression at stress genes (Fig.4). The following genes were selected based on a paper describing the identification of the INO80 complex in *S.cerevisiae*: Ies3 to 6, Taf14, Nhp10 and Arp5 (Shen et al. 2000). Wild type and mutant cells were treated with 0,4M NaCl for the indicated times and RNA was isolated. Transcript levels of the stress genes *CTT1* (Fig.5a) and *HSP12 (*Fig.5b*)* were determined by Northern Blotting. As mentioned before, a strain lacking the subunit Arp8 shows a hyperinduction phenotype at stress genes and was applied as a positive control.

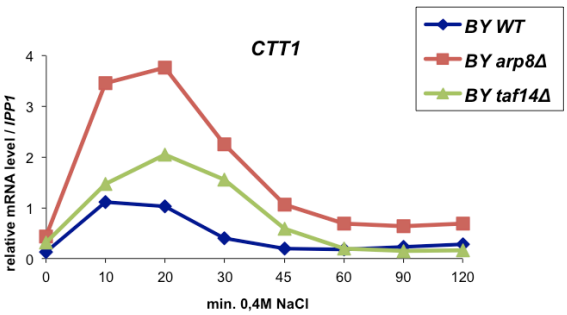
(a)


CTT1
BY WT
BY arp8Δ
BY ies3Δ
relative mRNA level / IPP1
min. 0,4M NaCl

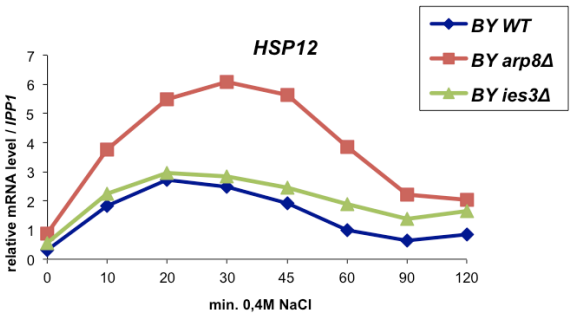

CTT1
BY WT
BY arp8Δ
BY ies4Δ
relative mRNA level / IPP1
min. 0,4M NaCl

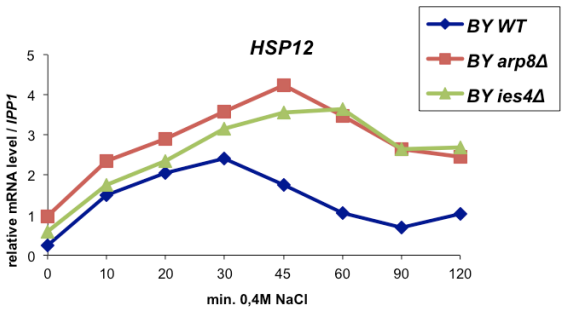

CTT1
BY WT
BY arp8Δ
BY ies5Δ
relative mRNA level / IPP1
min. 0,4M NaCl

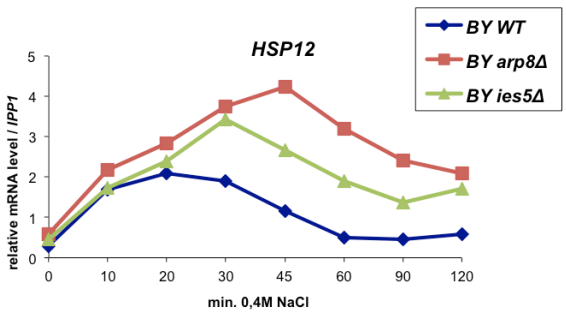

CTT1
BY WT
BY arp8Δ
BY ies6Δ
relative mRNA level / IPP1
min. 0,4M NaCl

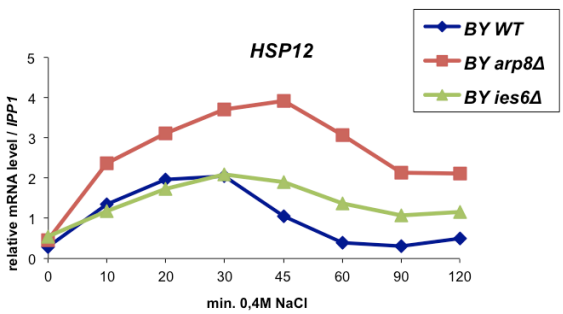

CTT1
BY WT
BY arp8Δ
BY taf14Δ
relative mRNA level / IPP1
min. 0,4M NaCl

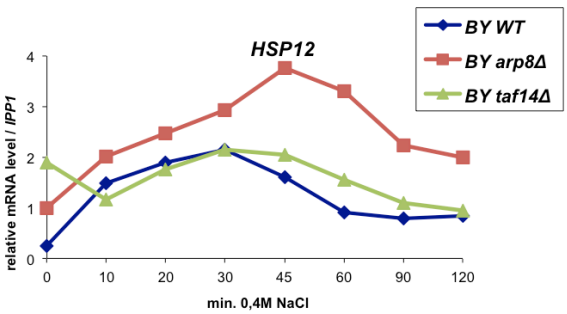
(b)


HSP12
BY WT
BY arp8Δ
BY ies3Δ
relative mRNA level / IPP1
min. 0,4M NaCl


HSP12
BY WT
BY arp8Δ
BY ies4Δ
relative mRNA level / IPP1
min. 0,4M NaCl


HSP12
BY WT
BY arp8Δ
BY ies5Δ
relative mRNA level / IPP1
min. 0,4M NaCl


HSP12
BY WT
BY arp8Δ
BY ies6Δ
relative mRNA level / IPP1
min. 0,4M NaCl


HSP12
BY WT
BY arp8Δ
BY taf14Δ
relative mRNA level / IPP1
min. 0,4M NaCl

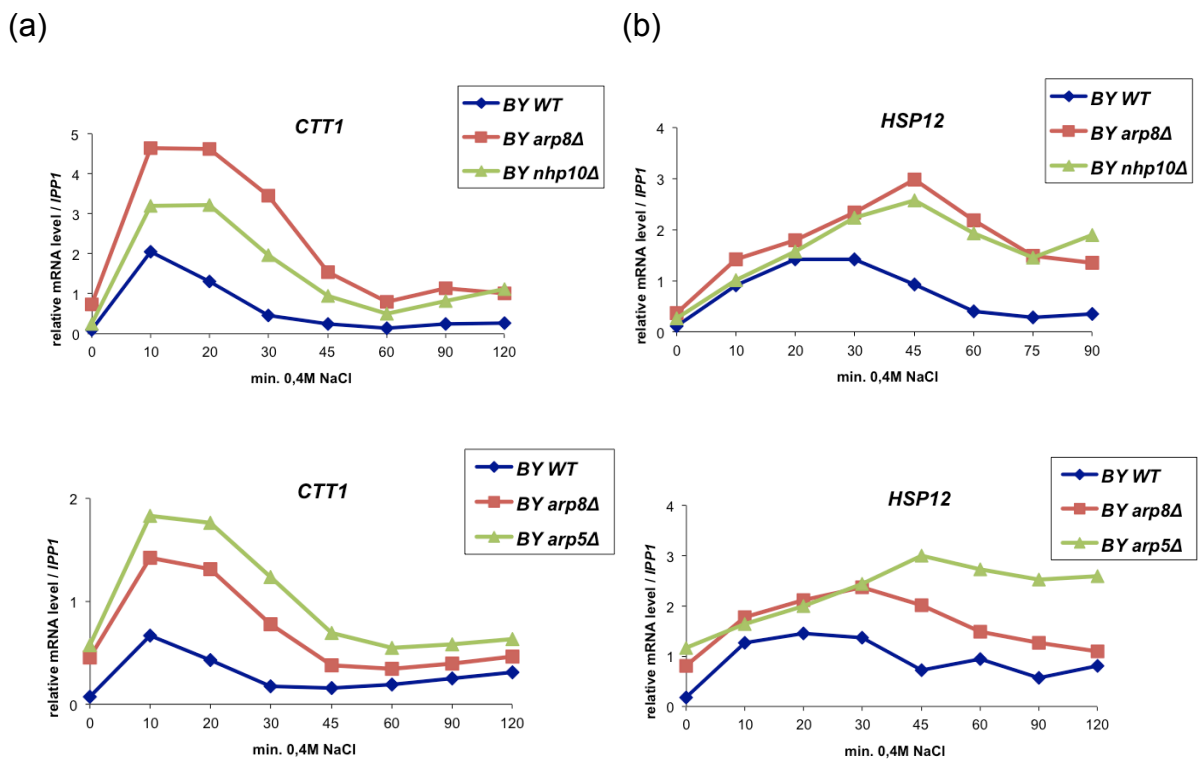


Figure 5. **(a)** *CTT1* and **(b)** *HSP12* transcript levels (relative to *IPP1*) of mutants of the INO80 complex (green line) compared to the reference strain (blue line). Cells were treated with 0,4M NaCl and RNA was isolated after 0, 10, 20, 30, 45, 60 and 90 min. An *arp8Δ* deletion strain was used as a positive control (red line).

Figure 5 illustrates that one of the tested mutants shows a similar phenotype as the *arp8Δ* mutant. Stress gene transcription is strongly increased in the *arp5Δ* mutant compared to the wild type and it is even slightly increased when compared to the *arp8Δ* mutant. In addition, it seems that basal transcript levels of *arp5Δ* mutants are elevated under non-induced conditions. This effect has also been observed at inducible loci in *arp8Δ* mutants (Görzer et al. 2003). The remaining strains (*ies3Δ, ies4Δ, ies5Δ, ies6Δ, nhp10Δ, taf14Δ*) exhibit minor increases in stress gene transcription.

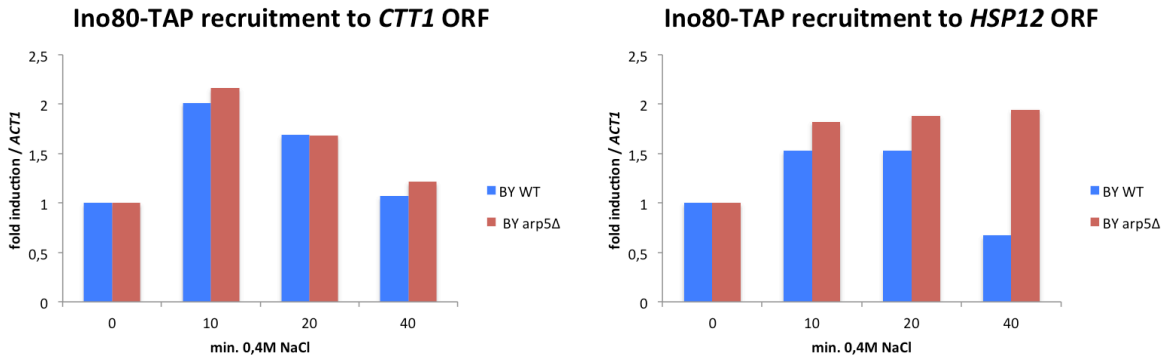
In summary, mutants of the non-essential subunits Arp5 and Arp8 as well as the essential Arp4 subunit show a strong hyperinduced transcription phenotype. As mentioned, Arp4 and Arp8 form a module with nuclear Actin, whereas Arp5 is integrated in the complex independent of these subunits (Shen et al. 2000; Fenn et al. 2011). This module is obviously a key player for stress gene repression by the INO80 chromatin remodeling complex. Apart from the Actin/Arp-module, also Arp5 has an important role in this respect. It remained to be determined whether these subunits could be required for recruitment of the complex. The remaining non-essential INO80 subunits tested in

this screen most likely do not have specific functions during stress gene transcription. Considering the function of the INO80 complex in other processes, one might speculate that these proteins are probably more important for INO80 mediated regulation of replication and repair of double strand breaks (Morrison et al. 2004). In addition, they might be favourable for complex stability which could explain the slightly increased transcript levels in mutants of the subunits Ies3 to 6, Nhp10 and Taf14 compared to the reference strain.

3.2 Arp5 is not essential for Ino80 Recruitment to Stress Genes

Nuclear Actin and the Actin related proteins Arp4, Arp5 and Arp8 were identified as key players for stress gene repression by the INO80 chromatin remodeler. I sought to determine whether these subunits are either important for recruitment or functionality of the complex. Therefore, a Chromatin Immunoprecipitation (ChIP) experiment was performed as described in the Materials and Methods section. The ChIP was done with a strain carrying a genomic C-terminal fusion of INO80 with a TAP (tandem affinity purification) -tag. The TAP-tagged version of Ino80 was detected at stress gene open reading frames in a wild type as well as an *arp8Δ* deletion strain. This experiment indicated that Arp8 is not essential for recruitment and might be more important for INO80 activity during active transcription (Klopf et al. 2009). Since deletion of Arp5 shows a similar or even stronger hyperinduced transcription phenotype compared to Arp8 deletions, we asked whether Arp5 might be the subunit mediating recruitment of the complex to chromatin. To answer this question, we analyzed Ino80-TAP recruitment to stress gene open reading frames in wild type and *arp5Δ* cells by ChIP (Fig.6).

(a)



(b)

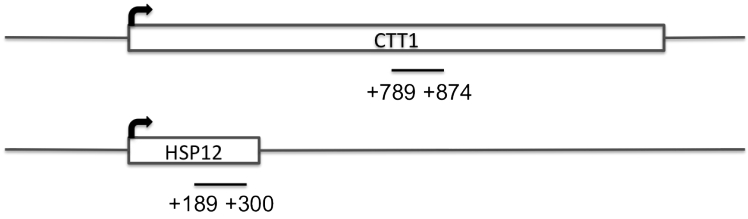


Figure 6. (a) Recruitment kinetics of Ino80-TAP to the coding region of *CTT1* and *HSP12*. Wild type and *arp5Δ* cells were treated with 0,4M NaCl for 0, 10, 20 and 40min. Ino80 bound DNA with was precipitated with anti- IgG antibody coated magnetic beads and quantified by qRT- PCR. **(b)** Positions of amplicons in the *CTT1*/*HSP12* ORF which were quantified by qRT-PCR.

As shown in figure 6, after treatment with high osmolarity stress Ino80-TAP recruitment to the open reading frame of *CTT1* is almost the same in the wild type and the *arp5Δ* deletion mutant. A similar recruitment kinetic can be observed at the *HSP12* gene in the first 20min after induction. However, after 40min Ino80 binding is reduced to basal level in the wild type whereas it is still bound in the *arp5Δ* mutant. We concluded that as already shown for the Arp8 subunit, Arp5 is not essential for recruitment of INO80 to stress genes. Hence, Arp5 plays a major role for functionality of INO80 at these loci.

3.3 The Ino80 ATPase is non-essential for Viability in the BY4741 background of *S.cerevisiae*

When I started with this work it was not clear if the Ino80 ATPase is essential for viability in the *S.cerevisiae* BY4741 strain background. The *ino80Δ* strain was not

available in the gene knock out collections and only the groups of X. Shen and H.J. Schüller reported deletion mutants lacking the *INO80* gene in different yeast backgrounds (Ebbert et al. 1999; Shen et al. 2000). For me it was important to obtain a BY4741 knockout of *INO80* to make further experiments comparable with previous data. Attempts to delete *INO80* with disruption constructs containing about 40bp of flanking regions and kanamycin-resistance or nourseothricin-resistance genes as selection markers (short flanking homology strategy) failed in the BY4741 reference strain. For that reason we assumed Ino80 to be essential for viability in a BY4741 background and tried to establish a conditional knock out system.

The anchor-away technique is used to create conditional yeast mutants allowing the removal of a target protein from the nucleus (Haruki et al. 2008). Therefore, the protein of interest is tagged with FRB (FKBP12-rapamycin-binding) in a strain containing a FKBP12- (FK506 binding protein) tagged anchor protein. The anchor is ribosomal protein (Rpl13A) which is synthesized in the cytosol, subsequently imported to the nucleus, assembled into ribosomes and then re-exported to the cytosol. Therefore the anchor shuttles between nucleus and cytosol. For assembly of the ribosomal subunits, the anchor protein is imported into the nucleus where it binds the FRB-tagged target protein in presence of rapamycin. After assembly of the ribosomal subunits it is transferred to the cytosol where translation is taking place and thereby anchors the target outside of the nucleus. The FRB domain is a component of the human mTOR protein and heterodimerizes with the FK506 binding protein in presence of rapamycin. After application of rapamycin the anchor protein interacts with FRB and rapamycin resulting in depletion of the nucleus from the target protein. To obtain a rapamycin inducible mutant of the Ino80 ATPase, I tagged the *INO80* gene with FRB in the anchor-away reference strain (HHY168) strain and confirmed the correct integration by PCR. Further, I tested growth of the positive Ino80-FRB clones on plates containing rapamycin (Fig.7).

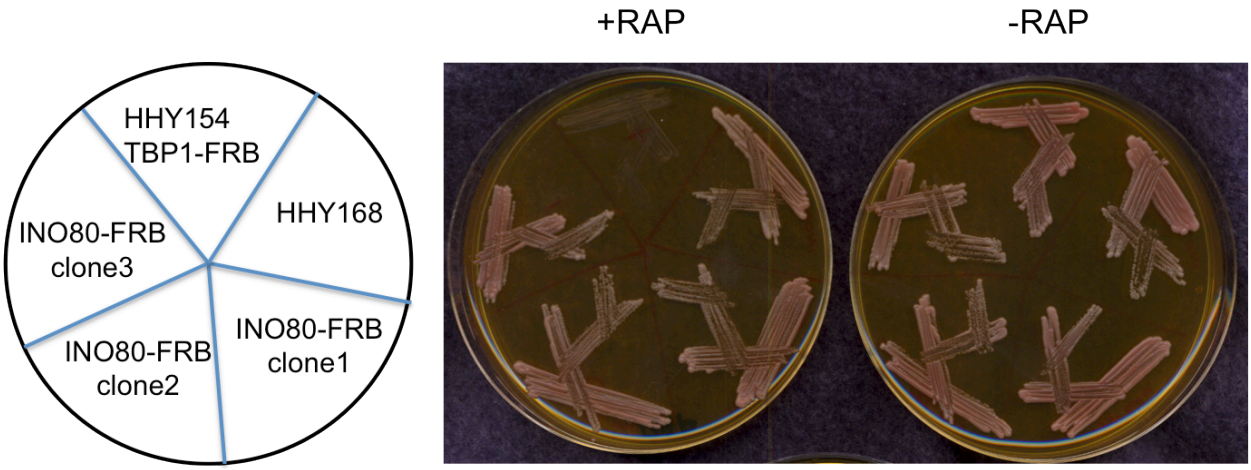


Figure 7. Rapamycin induced Ino80 depletion from the nucleus. Growth of yeast strains was tested on YEPD plates and plates containing 1µg/ml rapamycin. The INO80-FRB clones are viable on YEPD (-RAP) and rapamycin plates (+RAP) whereas the TBP1-FRB strain (HHY154) is unable to grow in the rapamycin induced situation.

As already mentioned we expected Ino80 to be essential for viability in yeast and consequently the INO80-FRB strain to be unable to grow on rapamycin plates. Figure 7 demonstrates growth of control strains and Ino80-FRB clones on rapamycin and YEPD plates. In the negative control strain HHY154 the *TBP1* gene, coding for TATAA-binding Protein (Tbp) which is a key factor of transcriptional initiation, is tagged with FRB. Solid medium supplemented with rapamycin (+RAP in Fig.7) lead to the removal of the essential Tbp protein from the nucleus and thus did not allow growth of the strain expressing the Tbp1-FRB fusion protein. In contrast, the same strain was able to grow normally on YEPD plates (-RAP in Fig.7) on which Tbp1 is not depleted from the nucleus. The positive control strain HHY168 of the anchor-away system does not express a protein fused to FRB. This strain was able to grow on both, +RAP and -RAP plates. These control strains demonstrated functionality of the anchor-away system as reported earlier and thus allowed screening of the Ino80-FRB transformants for rapamycin sensitivity (Haruki et al. 2008).

Surprisingly, all Ino80-FRB transformants were viable on rapamycin plates. We considered two possible explanations for this unexpected result. The first one was that the anchor away system is not suitable for the Ino80 protein. Since Ino80 is part of a 15-subunit complex it is possible that the FRB-tag is not accessible and Ino80 cannot be removed from the nucleus. The second possibility is that Ino80 is non-essential for viability in BY4741. Thereupon, I started another attempt to create a full knock out of Ino80 in the BY4741 strain. This time I transformed a disruption cassette containing

about 300bp of homologous *INO80* flanking sequences and the kanamycin-resistance gene. Hans Jörg Schüller kindly provided this construct on a plasmid (Ebbert et al. 1999). The BY4741 strain was transformed with the construct and obtained colonies were tested for correct integration by PCR. The group of C. Wu reported that BY4733 ino80Δ mutants are sensitive to hydroxyurea (HU), a toxin which is required for dNTP synthesis (Shen et al. 2000). To see if this phenotype is also observed in my transformants, I screened the positive clones for hydroxyurea sensitivity (Fig.8).

As shown in figure 8 the BY4741 wild type strain is capable to grow at a concentration of 100mM hydroxyurea. In contrast, the *ino80Δ* strain is more sensitive to hydroxyurea and unable to grow at these conditions. This sensitivity of the *ino80Δ* deletion mutant to hydroxyurea is complemented by plasmids expressing wild type Ino80 from its native promoter and from the strong *ADH1* promoter. The *ino80Δ* mutant phenotype cannot be complemented by plasmids expressing either an ATPase inactive mutant (K737R) or a 500bp 3´-terminal deletion of the *INO80* gene.

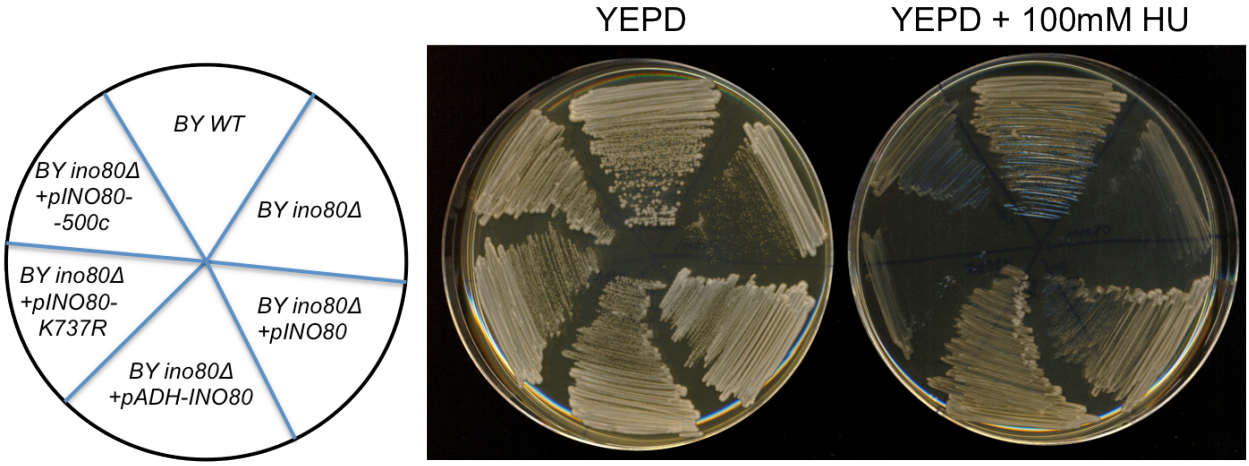


Figure 8. Growth of different yeast strains was observed on YEPD and YEPD + 100mM hydroxyurea plates. The BY wild type strain is resistant to hydroxyurea and able to grow at both conditions. The complete deletion of *INO80* as well as *Ino80Δ* strains expressing an ATPase inactive version (*pINO80-K737R*) or a 500bp c-terminal deletion (*pINO80-500c*) of Ino80 are sensitive to hydroxyurea and do not grow at these conditions. In contrast, expression of a wild type version of Ino80 (*pINO80*) and overexpression of the same protein (*pADH-INO80*) from a plasmid complements the growth phenotype of the *ino80Δ* mutant.

On YEPD plates, the *ino80Δ* deletion strain forms smaller colonies compared to the wild type which correlates with my observations in liquid medium where the *ino80Δ* strain exhibits a notable decrease of the vegetative growth rate. Taken together, a complete deletion of *INO80*, removal of 500bp of the 3´-terminus, and a single

nucleotide exchange leading to an ATPase inactivating K737R substitution increased sensitivity to hydroxyurea in the BY4741 strain.

The *INO80* deletion was further confirmed by Southern Blotting. Genomic DNA of a wild type and the Ino80 deletion was isolated and digested with appropriate restriction enzymes. Cut DNA fragments were separated by gel electrophoresis and transferred to a nylon membrane. One specific DNA fragment with varying size in wild type and mutant was detected using radioactive labelled DNA oligonucleotides (Fig.9).

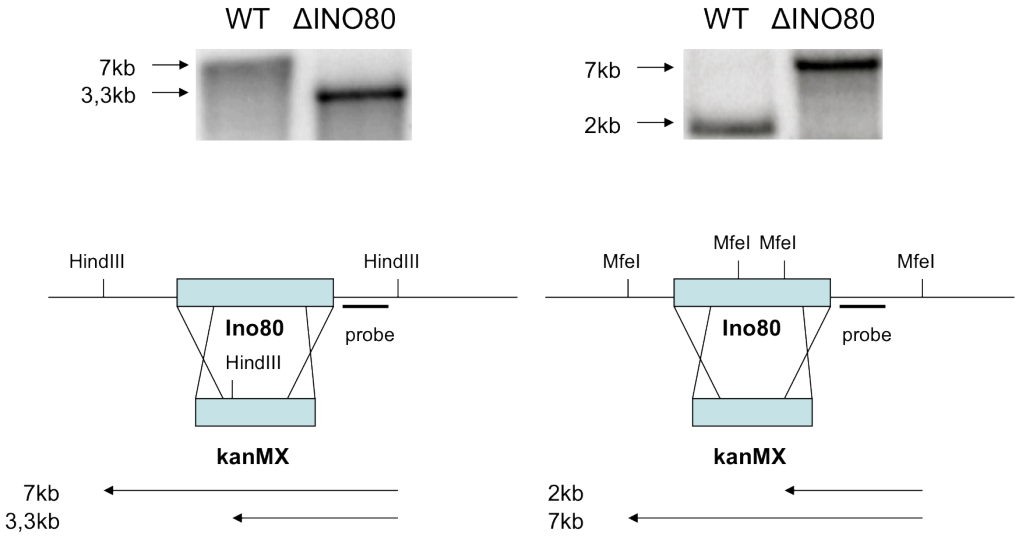


Figure 9. Genomic DNA of the BY4741 wild type and the BY4741 *ino80Δ* deletion strain was cut with either HindIII or MfeI. **(a)** HindIII digest resulted in a 7kb fragment in the wild type. An additional HindIII site within the kanamycin marker reduced the size of the fragment to 3,3kb in the *ino80Δ* mutant. **(b)** The MfeI digest resulted in a 7kb fragment in the *ino80Δ* mutant. Two additional MfeI sites within the *INO80* gene reduced the size of the fragment to 2kb.

Both digests prove the correct integration of the disruption cassette and the *INO80* gene to be successfully replaced by the kanamycin-resistance marker. The Ino80 gene is non-essential for viability in yeast and the knock out mutant was subsequently used to further examine the role of the Ino80 ATPase for transcription regulation.

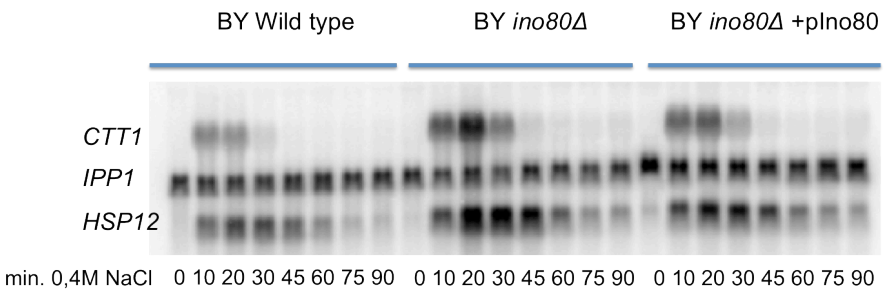
3.4 Deletion of the Ino80 ATPase causes a hyperinduced Transcription Phenotype comparable to deletions of Actin related proteins

So far it was shown that Arp5 and Arp8 are not essential for recruitment of the INO80 complex, however they exhibit a significant effect on stress gene transcript levels and it

seems that Arp5 and a module involving Arp4, Arp8 and nuclear Actin are more important for the chromatin remodeling activity during active transcription. At this point I was interested in the role of the Ino80 ATPase subunit. The ATPase subunit Ino80 harbours a split ATPase domain, which could provide energy for complex recruitment and/or chromatin remodeling. As illustrated in figure 3, the Ino80 subunit acts as a scaffold protein which is required for complex integrity and directly interacts with Actin related proteins of the INO80 complex (Bao and Shen 2007)). Considering these properties, I expected an important role of the Ino80 ATPase subunit for stress genes repression.

We monitored mRNA levels of the stress genes *CTT1* and *HSP12* in the wild type, the *ino80Δ* deletion strain and the *ino80Δ* deletion strain expressing a wild type version of Ino80 from a plasmid (Fig.10). Cells were grown to logarithmic phase (OD=0,8) and treated with a final concentration of 0,4M sodium chloride for times indicated in Figure 10.

(a)



(b)

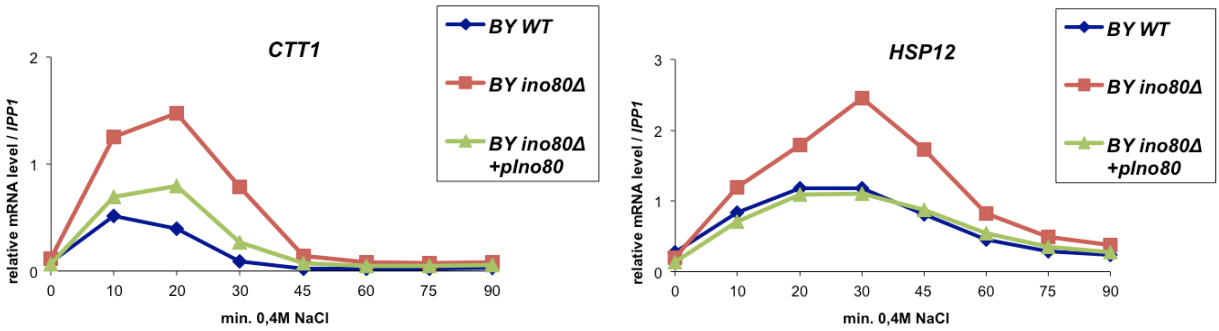


Figure 10. Raw data **(a)** and normalized values **(b)** of stress gene transcription kinetics of a BY wild type (blue line), BY Ino80Δ (red line) and a complemented BY Ino80Δ strain (green line) expressing Ino80 from a plasmid. Transcript levels of the *CTT1* and *HSP12* gene were determined 0, 10, 20, 30, 45, 60, 75 and 90min after induction by Northern Blotting.

Absence of the Ino80 ATPase resulted in hyperinduced and prolonged transcription (red lines) of *CTT1* and *HSP12* compared to the wild type (blue lines). The hyperinduction is comparable to the stress gene transcription phenotypes which were observed in strains lacking the Actin related proteins Arp4, Arp5 and Arp8. Expressing Ino80 from a plasmid complements the *ino80Δ* phenotype and shows a wild type like transcription kinetic (yellow lines). This experiment confirmed that the Ino80 subunit has an important role for transcriptional regulation of stress genes.

3.5 Ino80 ATPase activity is required for INO80 action at stress genes

Ino80 is the subunit acting as a scaffold protein, which could be important for complex assembly. I therefore asked if it is the ATPase activity of Ino80, which is responsible for the hyperinduced transcription in the Ino80 deletion mutant. I treated the *ino80Δ* deletion mutant expressing either a wild type version of Ino80 or an ATPase inactive mutant with 0,4M NaCl and measured transcript levels in a time scale experiment (fig 11). A plasmid carrying an *INO80-K737R* mutant was kindly provided by Hans Jörg Schüller (Ebbert et al. 1999).

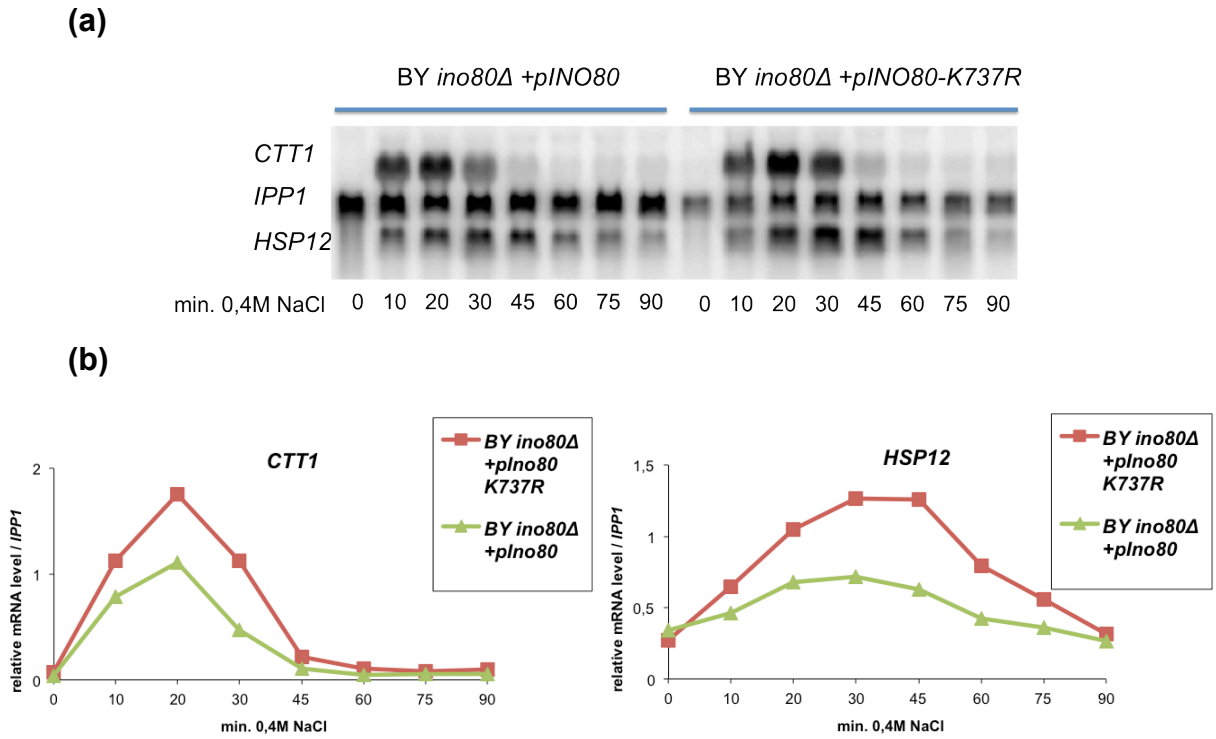


Figure 11. Raw data **(a)** and normalized stress gene transcript values **(b)** of the *ino80Δ* deletion strain expressing a wild type version and an ATPase inactive version of Ino80 (red line). The ATPase inactive mutant shows a hyperinduced transcription phenotype.

The strain expressing an ATPase inactive mutant of Ino80 exhibits a kinetic of stress genes which is almost identical to the *INO80* full knock mutant. This observation strongly suggests that ATP hydrolysis is the main activity of Ino80 for gene repression. Taken together it seems that a module of nuclear Actin, Arp4 and Arp8 as well as Arp5 and ATP hydrolysis by Ino80 are essential for stress gene repression mediated by INO80.

3.6 Ino80 and Arp8 are required for timely repositioning of stress gene promoter nucleosomes

To understand the role of these subunits on the level of chromatin, I examined positions and mobility of nucleosomes during active transcription by Nucleosome Scanning Assay (NuSA). NuSA is a powerful method to investigate nucleosome density at a single locus or even on a genome- wide scale. The main step of this technique is the isolation of the DNA fragments which are wrapped around nucleosomes and

thereby protected from MNase digest (Fig.13). Quantification of these DNA fragments correlates with nucleosome occurence at a specific locus.

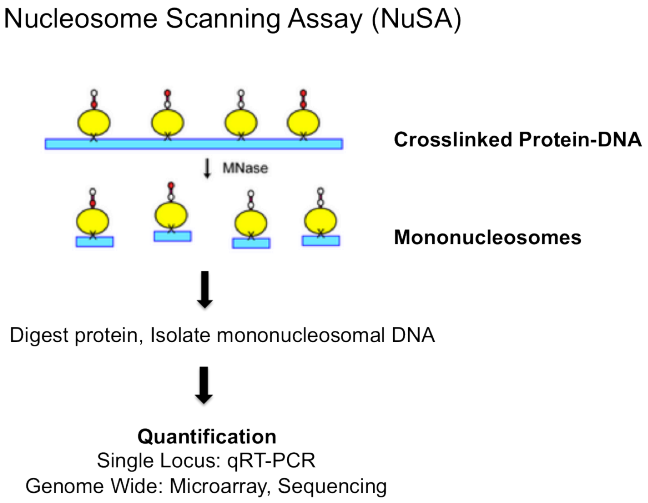
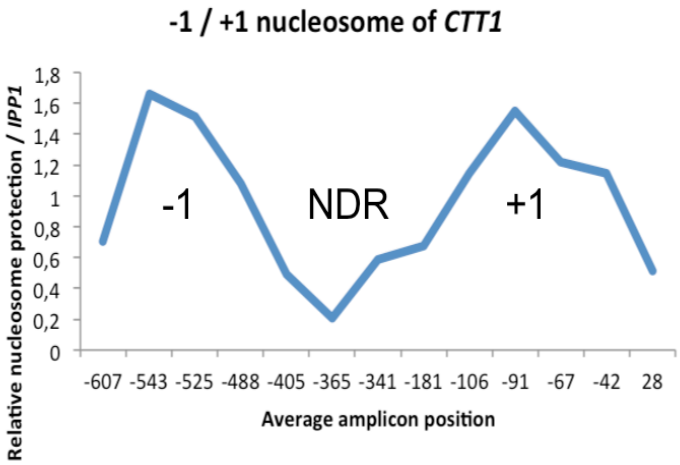


Figure 13. Schematic illustration of the Nucleosome Scanning Assay. Cells are crosslinked with formaldehyde and treated with Zymolyase. The spheroplasts contain the crosslinked Protein-DNA molecule which is digested with micrococcal MNase. DNA bound by a nucleosome is protected from Mnase digest. The mononucleosomes are further treated with proteinase to degrade protein, mononucleosmal DNA is purified and quantified. Figure 13 is adapted from (Liu et al. 2005)

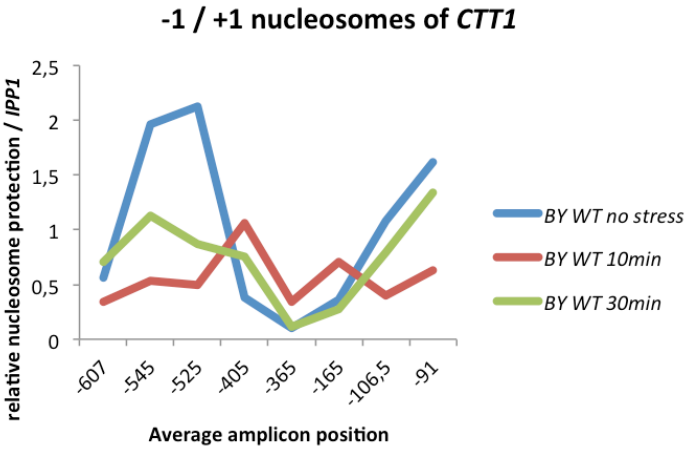
The promoter of almost every gene in yeast contains highly positioned -1 and +1 nucleosomes, which border a nucleosome-depleted region (NDR) (Jiang and Pugh 2009). Nucleosomes downstream of the +1 are less positioned. Our data for the *CTT1* gene show that the -1 and +1 nucleosomes are almost completely depleted 10min after stress induction. In a wild type strain these nucleosomes are partially repositioned after 30min whereas repositioning is strongly delayed in the *arp8Δ* deletion strain. Most notably, Arp8 mainly influences promoter nucleosomes, although it is recruited to the coding regions of stress genes (Eva Klopf, unpublished data).

The question was if deletions of *ARP8* and *INO80* behave similar on the level of chromatin during active transcription. I treated wild type, *arp8Δ* and *ino80Δ* strains with 0,4M NaCl and analyzed promoter nucleosome dynamics over time by NuSA (Fig.14).

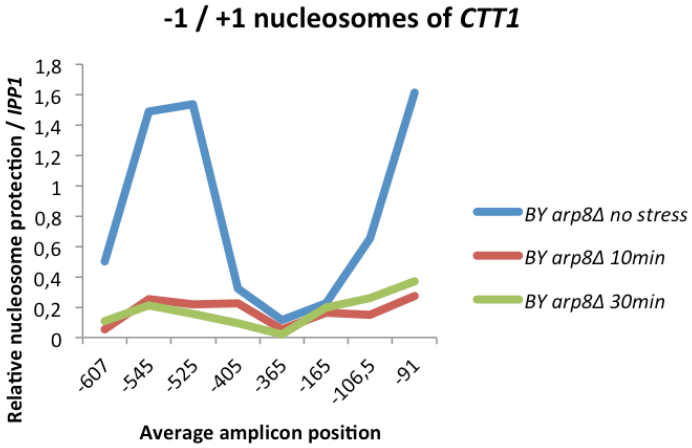
(a)


-1 / +1 nucleosome of CTT1
Relative nucleosome protection / IPP1
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
-1
NDR
+1
-607 -543 -525 -488 -405 -365 -341 -181 -106 -91 -67 -42 28
Average amplicon position

(b)


-1 / +1 nucleosomes of CTT1
relative nucleosome protection / IPP1
2,5
2
1,5
1
0,5
0
-607
-545
-525
-405
-365
-165
-106,5
-91
Average amplicon position
BY WT no stress
BY WT 10min
BY WT 30min

(c)


-1 / +1 nucleosomes of CTT1
Relative nucleosome protection / IPP1
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
-607
-545
-525
-405
-365
-165
-106,5
-91
Average amplicon position
BY arp8Δ no stress
BY arp8Δ 10min
BY arp8Δ 30min

(d)

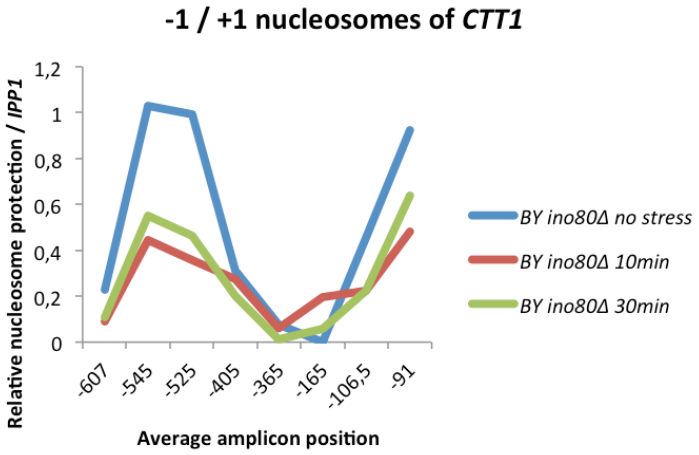


Figure 14. (a) Schematic illustration of the *CTT1* nucleosome depleted region (NDR) flanked by -1 and +1 promoter nucleosomes. *CTT1* Promoter nucleosomes dynamics of a BY wild type **(b)**, *arp8Δ* **(c)** and *ino80Δ* **(d)** strain are shown at non-induced conditions and after treatment with 0,4M NaCl.

As shown in Figure 14, *CTT1* promoter nucleosomes of the wild type and the *arp8Δ* mutant are completely depleted 10min after stress application. In contrast, promoter nucleosomes of the *ino80Δ* strain are not completely removed after 10min suggesting that lack of the Ino80 subunit could diminish promoter depletion.

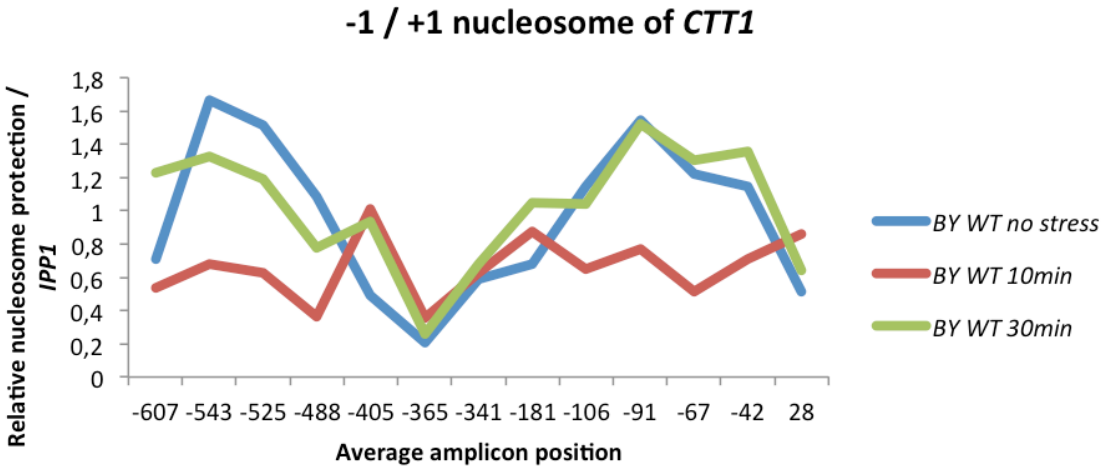
Wild type nucleosomes are partially repositioned after 30min whereas nucleosomes of the *arp8Δ* strain are still depleted and reconstitution is delayed. It seems that *ino80Δ* strain shows a nucleosome repositioning kinetic which is similar to the *arp8Δ* mutant. Promoter nucleosomes are almost at the same level after 10min and 30min indicating that repositioning of promoter nucleosomes is also delayed in the *ino80Δ* mutant. A second independent experiment showed a similar effect at the -1 nucleosome. After 30minutes, the wild type nucleosome is repositioned whereas the nucleosome of *ino80Δ* strain is still depleted. (Fig.15).

3.7 Positioning and depletion of nucleosomes stress gene promoters is reduced *ino80Δ* mutants.

The *ino80Δ* strain reveals two interesting observations regarding promoter nucleosome mobility during active transcription. First of all, promoter nucleosome depletion after 10min is markedly reduced (Fig.14) and this effect is not observed in the

arp8Δ mutant. A similar effect was observed in a second, independent experiment in which promoter nucleosome dynamics of the wild type and *ino80Δ* strain were compared (Fig.15).

(a)



(b)

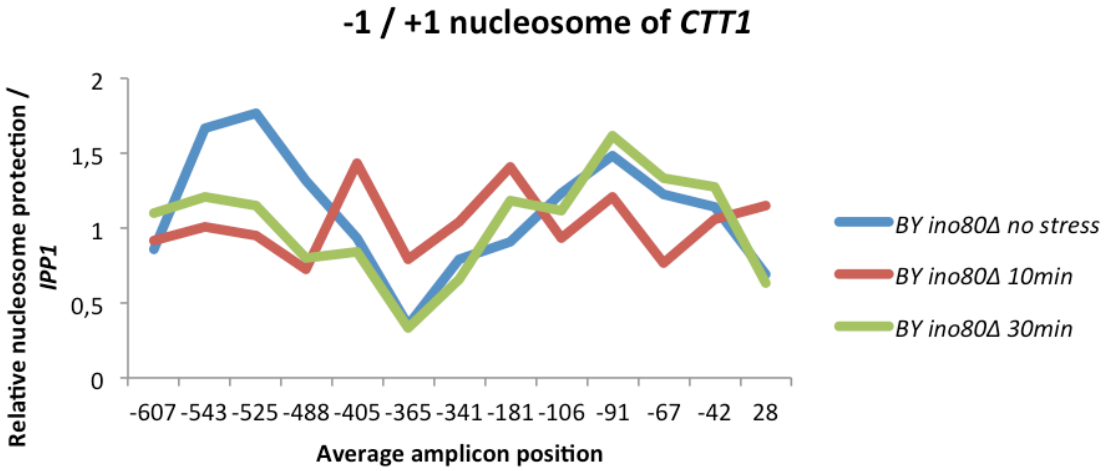


Figure 15. Dynamics of the -1 and +1 nucleosomes of *CTT1* during stress gene transcription. BY wild type **(a)** and BY *ino80Δ* **(b)** cells were treated with hyperosmolarity stress and *CTT1* promoter nucleosomes were analyzed by NuSA. Obviously, promoter nucleosome depletion after 10min is notably reduced in the *ino80Δ* mutant.

Eviction of promoter nucleosomes seems to be diminished in *ino80Δ* compared to *arp8Δ* strains which raised the question if the Ino80 ATPase could be required for an additional function of INO80 during stress gene transcription.

A second observation was that in *arp8Δ* and *ino80Δ* strains, the -1 nucleosome levels are reduced compared to the wild type (Fig.16).

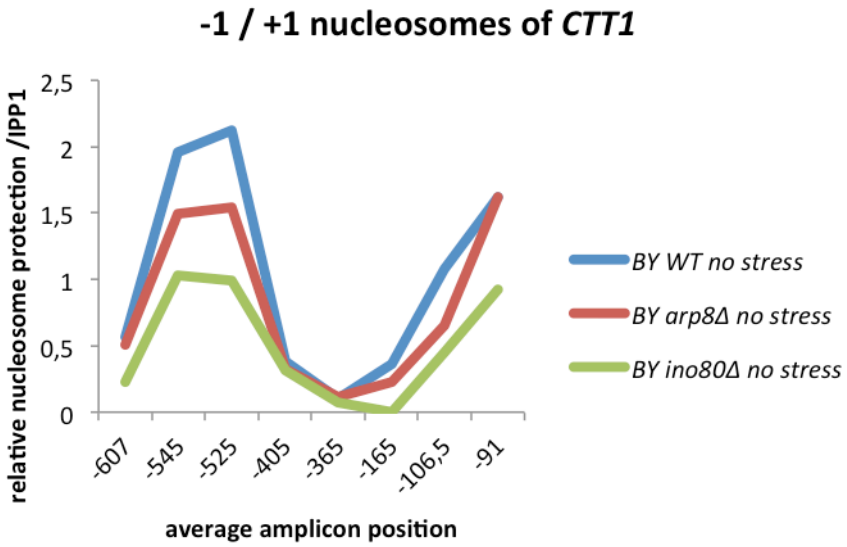


Figure 16. Promoter nucleosome positions of wild type, arp8Δ and ino80Δ strains at non- induced conditions. Both mutants of the INO80 complex show significant reduction of the -1 nucleosome peak.

This effect was observed in several independent experiments for the -1 and +1 nucleosome in *arp8Δ* mutants (Eva Klopf, unpublished data) and could indicate a role of INO80 for promoter nucleosome positioning. Further work might elucidate the apparent contradiction of the chromatin localization of INO80 to the open reading frame and the striking effects on the promoter nucleosomes.

4. Discussion

Yeast cells respond to extracellular stress conditions through induction of a set of so-called stress genes. This highly dynamic transcriptional process is tightly regulated by transcriptional activators and co-factors such as histone modifiers and chromatin remodelers. The importance of these factors for transcriptional regulation varies at different loci. Some are required for assembly of the pre-initiation complex (PIC) and thus essential for activation. Other factors are not essential for the formation of the PIC, however, they are involved in fine-tuning of the transcriptional response.

The main results from this work are the following observations:

- The Actin/Arp-module and Arp5 are key players for INO80 action at stress genes.
- Arp5 is not essential for Ino80 recruitment to stress genes.
- The Ino80 ATPase subunit is non-essential for viability in the BY4741 background of *S.cerevisiae.*
- Deletion of the Ino80 ATPase subunit causes a hyperinduced transcription phenotype comparable to deletions of Arp5 and Arp8.
- Ino80 ATPase activity is required for INO80 action at stress genes.
- Ino80 is required both for positioning and efficient depletion of promoter nucleosomes.

The INO80 Actin/Arp-module and Arp5 are key players for INO80 mediated transcriptional repression of stress genes

The INO80 complex consists of 15 subunits and has been shown to be a negative regulator of transcription of highly induced genes. A module of nuclear Actin, Arp4 and Arp8 as well as the Arp5 subunit are key components required for INO80 action at stress genes. Strains lacking one of these subunits exhibit hyperinduced and prolonged transcription. This results in dramatically increased mRNA levels of stress genes. Functionally impaired INO80 complexes could be responsible for the hyperinduced transcription phenotypes observed in these mutants. Nevertheless, the possibility remains that this effect could be caused by a recruitment defect preventing the interaction of the complex with chromatin. Therefore, all key subunits were designated as candidates for being factors mediating recruitment of INO80 to stress genes.

Recruitment of the Ino80 ATPase is independent of Actin related proteins

INO80 is targeted to coding regions of stress genes where it acts as a co-factor for transcriptional repression (Klopf et al. 2009). The recruiting mechanism for INO80 during active transcription is currently unknown. The phosphorylated form of H2AX (designated γ-H2AX) mediates recruitment of INO80 to double strand breaks (Morrison et al. 2004). However, γ-H2AX is not required for recruitment of INO80 to transcription sites. Furthermore, histone tail methylations by Set1/2 have been excluded to mediate recruitment of INO80 (Klopf et al. 2009). As already described previously, the Ino80 ATPase is targeted to stress genes after induction and this recruitment is still observed in a strain lacking Arp8 indicating that Arp8 is not essential for recruitment of the complex (Klopf et al. 2009). These observations also suggest that nuclear Actin and Arp4 could not be exclusively necessary for the recruitment mechanism because incorporation of these subunits into the INO80 complex require Arp8.

For that reason I suspected Arp5 to be the main subunit mediating recruitment of the INO80 complex to actively transcribed genes. As shown in figure 6, Ino80 is still detectable at the open reading frame of the *CTT1* gene in an *arp5Δ* deletion strain. This observation suggests that Arp5 is not essential for Ino80 recruitment. The possibility remains that Arp8 and Arp5 act redundantly for Ino80 recruitment. In addition, both might be required for functionality of the INO80 complex.

The Ino80 ATPase is targeted to sites of active transcription independent of the Arp8 and Arp5. However, nuclear Actin and Arps are found in many chromatin reconstructing complexes and possibly developed an independent recruitment mechanism. Interestingly, recent structural data indicate that the acidic loop insertions of Arp8 are responsible for interaction with histones (Fenn et al. 2011).

The Ino80 ATPase is a key component for stress gene repression by INO80

Here I confirmed that Ino80 is not essential for viability in the BY4741 background of *S.cerevisiae*. The *ino80Δ* deletion causes a hyperinduced transcription phenotype of stress genes which is comparable to deletions of the Actin related proteins Arp5 and Arp8 as well as a temperature sensitive $arp4^{G161D}$ mutant. The same effect is observed in a strain expressing an ATPase inactive mutant of Ino80 indicating that ATP hydrolysis is essential for stress gene repression. These data suggest that in addition to the Actin/Arp-module and Arp5, Ino80 is also a key component for INO80 action at stress genes and a promising candidate for mediating recruitment of the complex. As described above, I also found that the Ino80 ATPase is recruited to sites of active transcription independent of Arp5 and Arp8. From these observations I assumed that Actin/Arp-module and the remaining complex including the Ino80 ATPase and Arp5 could be recruited to stress genes independently of each other. To further investigate this presumption, one might examine if the Actin/Arp-module is recruited independent of the INO80 ATPase. Figure 17 summarizes a possible future workflow to answer the open questions regarding the role of the Ino80 ATPase subunit for recruitment of the Actin/Arp-module. First, binding of Arp8-TAP at stress genes could be tested in an *ino80Δ* deletion strain.

If the Arp8 subunit can be detected at stress gene ORFs in an *ino80Δ* deletion background, the Actin/Arp-module would target transcription sites independent of Ino80. Also, ATPase activity of Ino80 would not be essential for targeting supporting a model in which the Actin/Arp-module and Ino80 can be recruited to chromatin independent of each other. In case that Arp8 could not be detected at stress gene ORFs in an *ino80Δ* mutant, Ino80 would be essential for recruitment of the Actin/Arp-module. There are two different ways of how Ino80 could be involved in targeting the Actin/Arp-module to sites of active transcription: i) Ino80 either directly interacts with chromatin or ii) Ino80

indirectly contributes to the recruitment mechanism by providing energy via its ATPase activity. Another indirect effect could be that Ino80 influences recruitment because it is required for integrity of the complex. To approach this question one could measure recruitment of Arp8 in the *ino80Δ* deletion background expressing an ATPase inactive version of Ino80. The advantage of the ATPase mutant in contrast to the full deletion is that Ino80 is physically available and direct interactions of protein and chromatin structures are still possible. Again there are two possible scenarios: Recruitment of Arp8 in a strain expressing the ATPase inactive Ino80 ATPase would suggest that Ino80 directly interacts with chromatin. In case that Arp8 could not be detected in an ATPase inactive mutant, Ino80 could indirectly contribute to the recruitment mechanism by providing energy via ATP hydrolysis.

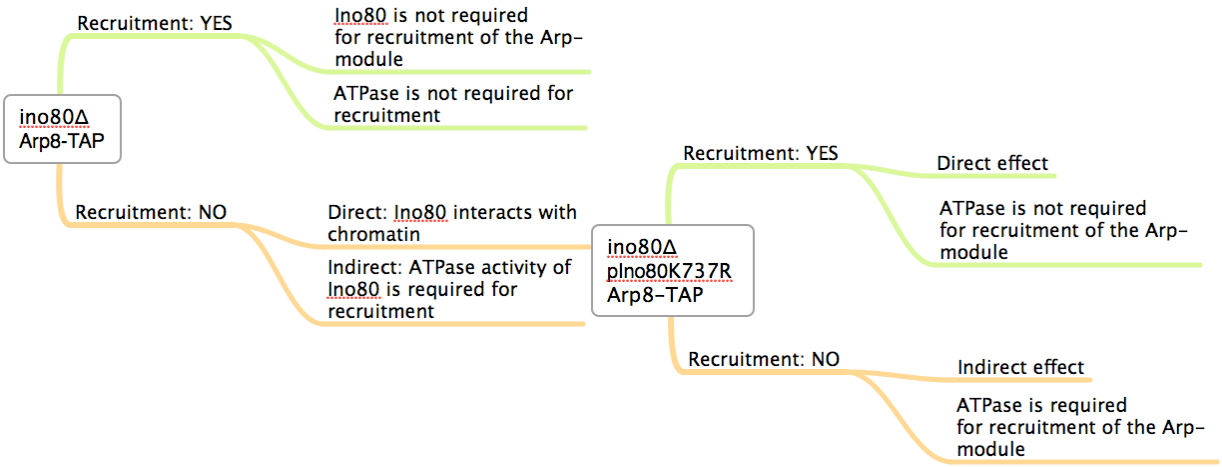


Figure 17. Mindmap of future experiments to identify the role of the Ino80 ATPase for recruitment of INO80 to stress genes.

The Ino80 ATPase is involved in reconstitution of evicted nucleosomes

The repositioning of promoter nucleosomes after induced transcription of stress genes is significantly delayed in *arp8Δ* and *ino80Δ* mutants. These observations indicate that INO80 is required for reconstitution of promoter nucleosomes and raised the question why the INO80 complex is recruited to the ORF regions but strongly affects nucleosomes in the promoter of stress genes. INO80 could act as a bridging factor to mediate promoter nucleosome repositioning. The complex could also be involved in the recruitment of other chromatin reconstructing factors which act at the promoter. Contrary, it would be thinkable that Ino80 is actually present at the promoter but cannot

be detected for technical reasons due to low abundance or weak and transient interaction.

Ino80 ATPase and nucleosome depletion

Although deletions of the Ino80 ATPase and Arp8 show a similar phenotype at the level of stress gene transcription, nucleosome dynamics are not identical in the two mutants. The *ino80Δ* mutant exhibits reduced promoter nucleosome depletion after induction of the *CTT1* gene. This effect is not observed in the *arp8Δ* mutant and could indicate an Arp8 independent role of the INO80 complex at promoters of stress genes. We can draw a model in which the INO80 complex is required for two different functions at stress genes (Fig.18). Repositioning of nucleosomes after RNA Pol II is associated with a repressive effect for transcription. This function is impaired in strains lacking Ino80 and/or Arp8. The second function of the Ino80 ATPase could be a role for promoter nucleosome depletion after induction. INO80 had been associated with transcriptional upregulation at the *PHO* and *INO1* loci (Ebbert et al. 1999; Barbaric et al. 2007; Ford et al. 2007).

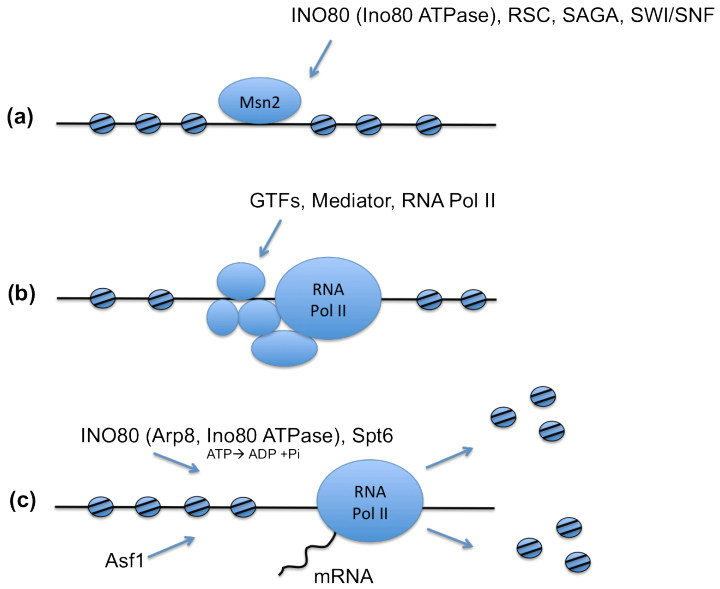


Figure 18. Simplified model of chromatin remodeling activities during stress gene transcription. **(a) (b)** The transcriptional activator Msn2 binds so stress response elements (STREs), co-factors SAGA and SWI/SNF and probably INO80 are recruited resulting in promoter nucleosome depletion and binding of general transcription factors (GTFs), Mediator and RNA Pol II. **(c)** During elongation promoter and ORF nucleosomes are repositioned after transcribing RNA Pol II. Repositioning is accomplished by a mechanism involving Actin related proteins and the ATPase subunit of INO80 as well as the histone chaperones Spt6 and Asf1.

After binding of the transcriptional activator Msn2, co-factors such as SWI/SNF and SAGA are targeted to stress genes to cooperate for the depletion of the promoter region (Fig.18a, b). Promoter depletion involves several types of chromatin reconstructing factors such as chromatin remodeler, histone modifiers and histone chaperones and could also include the INO80 complex. Subsequent binding of GTFs, Mediator and RNA Pol II assembles the pre-initiation complex and the transcriptional machinery proceeds into productive elongation. INO80 is still associated to RNA Pol II or coding regions of stress genes and facilitates repositioning of nucleosomes behind RNA Pol II (Fig.18c). Therefore INO80 cooperates with the histone chaperone Spt6 which acts in the same pathway. In addition the histone chaperone Asf1 contributes to nucleosome reconstitution in a parallel pathway (Klopf et al. 2009).

It seems that Arp8 is required for efficient assembly of nucleosomes back to their original positions behind transcribing RNA Pol II. However, one might speculate that nucleosome depletion represents an additional role of INO80 which could be provoked by the Ino80 ATPase and other subunits of the complex. INO80 is probably involved in maintaining a balance between activation and repression. It seems that the position of this balance can be shifted to the activating as well as the repressing side depending on the characteristics of a particular locus. One possibility of how INO80 may influence nucleosome depletion is the removal of H2A.Z from the promoter nucleosomes. To test whether reduced nucleosome depletion in *ino80Δ* mutants is connected with H2A.Z, nucleosome depletion could be examined in an *ino80Δ*, *htz1Δ* double mutant. The phenotype should disappear in the double mutant in case that reduced nucleosome depletion correlates with H2A.Z removal by INO80.

INO80 ATPase and nucleosome positioning

Finally, we observed decreased levels of the -1 and +1 nucleosomes in the *ino80Δ* mutant at non-induced conditions (Fig.16). This phenotype was also shown in *arp8Δ* deletion mutants in several independent NuSA experiments (Eva Klopf, unpublished data). Decreased promoter nucleosome peaks could be an indirect result of increasing RNA Pol II occurrence at the promoters of INO80 dependent genes. This would correlate with recent genome wide transcription data indicating that basal transcript levels of many genes are upregulated in *arp8Δ* mutants (Klopf et al. 2009). To revise this presumption one could turn down basal expression levels of Msn2/4 to inhibit

transcription at the *CTT1* gene. If promoter nucleosome levels correlate with RNA Pol II occurrence at the promoter, they should return to wild type levels in a strain lacking Ino80 when transcription is inhibited. If nucleosome levels are still decreased in the double mutant, the INO80 complex could feature an additional function at stress genes for appropriate positioning of promoter nucleosomes.

In summary, the INO80 complex participates in transcriptional regulation of many genes in *S.cerevisiae.* Most of them are strongly induced and INO80 is important for the fine-tuning of the transcriptional output. A module of ADP-bound nuclear Actin, Arp4 and Arp8 seems together with Arp5 and the Ino80 ATPase responsible for INO80 mediated repression of stress genes by reconstitution of evicted promoter and most likely ORF nucleosomes. Unfortunately I could not find a single subunit mediating recruitment of INO80 complex to sites of active transcription. The most promising candidates were the Actin related proteins of the INO80 complex. Our data suggest that none of them are essential for recruitment of the Ino80 ATPase. In combination with recent structural data of Arp4 and Arp8 which indicate that both interact with histones, I concluded that the INO80 complex could be recruited to stress genes in two different parts (Fenn et al. 2011). The Actin/Arp-module as well as the Ino80 ATPase together with the additional INO80 subunits could be targeted to stress genes independent of each other. This model should be further investigated and could help to answer the questions on how modules of nuclear Actin and Arps regulate transcription as integral components of many different chromatin reconstructing complexes.

5. Materials and Methods

Yeast strains

Strain	**Genotype**	**Reference**
BY4741	*MATa; his3Δ; leu2Δ ; met15Δ; ura3Δ*	Euroscarf
BY *ies3Δ*	*MATa; ies3Δ::kanMX4 (isogenic to BY4741)*	Euroscarf
BY *ies4Δ*	*MATa; ies4Δ::kanMX4 (isogenic to BY4741)*	Euroscarf
BY *ies5Δ*	*MATa; ies5Δ::kanMX4 (isogenic to BY4741)*	Euroscarf
BY *ies6Δ*	*MATa; ies6Δ::kanMX4 (isogenic to BY4741)*	Euroscarf
BY *nhp10Δ*	*MATa; nhp10Δ::kanMX4 (isogenic to BY4741)*	Euroscarf
BY *arp5Δ*	*MATa; arp5Δ::kanMX4 (isogenic to BY4741)*	Euroscarf
BY *arp8Δ*	*MATa; arp8Δ::kanMX4 (isogenic to BY4741)*	Euroscarf
BY *taf14Δ*	*MATa; taf14Δ::kanMX4 (isogenic to BY4741)*	Euroscarf
BY *ino80Δ*	*MATa; ino80Δ::kanMX4 (isogenic to BY4741)*	this study
BY *ino80Δ pINO80*-TAP	*MATa; ino80Δ::kanMX4 (isogenic to BY4741) [pFAG Ino80-TAP]*	this study
BY *ino80Δ pINO80-K737R*-TAP	*MATa; ino80Δ::kanMX4 (isogenic to BY4741) [pFAG Ino80K737R-TAP]*	this study
BY Ino80-TAP	MATa; his3*Δ*; leu2*Δ*; met15*Δ*; ura3*Δ* Ino80-TAPNatMX	Open Biosystems
BY *arp5Δ* Ino80-TAP	*MATa; arp5Δ::kanMX4 (isogenic to BY4741)* Ino80-TAPNatMX	this study
HHY168	*MATalpha tor1-1 fpr1::NAT RPL13A-2×FKBP12::TRP1*	Euroscarf
HHY154	*MATalpha tor1-1 fpr1::NAT RPL13A-2xFKB12::TRP1 TBP1-FRB::kanMX6*	Euroscarf

Table 1. List of yeast strains used in this work

Primer list

Primer	***Sequence 5'→3'***
CTT1_ORF2_for	*AATCAGGCAAAGCTGTTCACTC*
CTT1_ORF2_rev	*ACATTCTTCGTTAGGGTGATGG*
HSP12_for	*CAAGGATAACGCTGAAGGTCAAG*
HSP12_rev	*GACACGACCGGAATATTCG*
ACT1_for	*ATTAACAATGGATTCTGGTATGTG*
ACT1_rev	*GGTAAAAGAGAAATCTCTCGAGCA*

Table 2. List of primers used for ChIP experiments

Plasmid Cloning

All plasmids were created by standard cloning methods. Plasmids and inserts were cut with restriction enzyme/s for 4h. Single cut plasmids were treated with thermosensitive alkaline phosphatase (TSAP, Promega) in the restriction buffer for 30min. TSAP treated plasmids were incubated at 65°C for 10min to stop the reaction. Plasmids and inserts were loaded on an agarose gel and separated bands were cut out and eluted. Ligations were performed according to manufacturer's recommendation using 3 µl of vector DNA and variable amounts of insert DNA including a negative control without insert DNA. Ligation reactions were heat shock transformed into competent *E.coli* TOP10. Therefore, 50µl of bacteria were mixed with 5µl ligation reaction, incubated on ice and heat shocked for 90sec on 42°C. Cells were cooled down on ice and incubated in 500µl LB at 37°C for 1h.

Ino80 plasmids were derived from plasmid pAdh1-Msn2-GFP (Görner et al. 1998) by exchange of Msn2-GFP with Ino80 (pADH-INO80) further introduction of tandem affinity purification (TAP) tag (pADH-INO80-TAP) and exchange of the ADH promoter by a native Ino80 promoter (pINO80-TAP). Plasmid pINO80-K737R-TAP was created by replacement of a 2300bp fragment from the Ino80 gene of pINO80-TAP with the same fragment of an Ino80K737R construct and confirmed by sequencing. The initial Ino80K737R plasmid was a gift from Dr. H.J. Schueller (Ebbert et al. 1999).

Preparation of competent *E.coli* TOP10

100ml LB medium was inoculated with 5ml of a o/n culture of E.coli TOP10 cells and grown to OD=0,4. Cells were transferred into 2 50ml tubes and incubated on ice for 10min. Cells were centrifuged for 10min at 4000rpm/4°C and the pellet was resuspended in 30ml of cold buffer1 (80mM $MgCl_2$, 20mM $CaCl_2$). Cells were again centrifuged for 10min at 4000rpm/4°C and the pellet was 2ml of ice cold buffer2 (100mM $CaCl_2$, 10% glycerol). The *E.coli* TOP10 cells were frozen in liquid nitrogen, divided into 100µl aliquots and stored at -80°C.

Plasmid transformation (*E.coli*)

Transformation of a Ligation: 50µl competent cells were mixed with 5µl ligation reaction and incubated on ice for 30min, followed by a 90s heat shock at 42°C. Cells were incubated on ice for 5min, 500µl LB medium was added and shaken at 37°C for 1hour. Cells were plated on agar containing 100µg/ml ampicillin or 50µg/ml carbenicillin.

Yeast transformation

Over night cultured cells were diluted to OD=0,2 and grown to OD=0,5. 50ml cells were centrifuged for 3min at 2200rpm, supernatant was removed and pellet was washed with 25ml autoclaved H2O. The pellet was resuspended in 1ml 100mM LiAc, transferred to 2ml tubes, supernatants were removed and pellets were resuspended in 300µl 100mM LiAc. 50µl cells were centrifuged, the supernatant was removed and 240µl 50% PEG 3350, 50µl ssDNA (boiled and incubated on ice) and 36µl 1M LiAc were added. 10µl H2O and 15µl disruption cassette were added (25µl H2O for negative control) and strongly mixed for 1min. Cells were incubated (gently shaken) for 30min on 30° followed by heat shock for 20min on 42°C. Further, pellets were resuspended in 800µl YPD, incubated for 2,5h at 30°C and plated on plates containing the appropriate antibiotics. Cells which were transformed with an auxotrophy marker were directly plated on medium lacking the aminoacid after heat shock.

Plasmid transformation (*S.cerevisiae*)

200µl plasmid transformation buffer was strongly mixed with 1µl plasmid and yeast cells grown on agar. Cells were incubated on 45°C for 45min. After centrifugation, pellets were resuspended in 200µl autoclaved H2O and plated on selective medium.

Northern Blot

Over night cultured cells were diluted to OD=0,2 (0,22 for strains with reduced growth phenotype) and grown to OD=0,7. Complete cultures were treated with 0,4M NaCl and after different periods of time 30ml samples were centrifuged at 2200rpm for 2min,

supernatants were removed and the pellets were transfer into screw cap tubes. Again supernatants were removed, cell pellets were frozen in liquid nitrogen and stored at -20°C. During RNA extraction, all centrifugation steps were performed at 14000rpm in the cold room. 200µl RNA extraction buffer, 200µl phenol and glass beads were added to the samples and cells were broken with a Fast Prep for 2x10s at 4°C at speed 6 (alternatively 2x10min on a Vibrax , full speed). Cell suspensions were centrifuged for 10min and the upper aqueous layer was transferred to 1,5ml tubes and an equal volume of chloroform was added. After the last step was repeated, the aqueous phase was transferred into 1,5µl tubes containing 1/20 volume of 3 to 4M NaAc pH 4,2 / 2 volumes EtOH abs, inverted and stored at -20°C for 30min. Precipitated RNA was centrifuged for 10min, supernatant was removed and the pellet was washed in 200µl 80% EtOH. Supernatants were completely removed and dry pellets were resuspended in 50µl DEPC water. RNA concentrations were determined by measurerment of A260nm and 15µg total RNA was loaded on a gel. Therefore absorbance of 2,5µl RNA in 1ml DEPC water was measured at 260nm. The concentration in µg/µl (A260*16) and the amount of RNA which is required for 15µg total RNA (15/Conc[µg/µl]*2) was calculated. Remaining volumes to 9µl were filled up with DEPC water, 31µl FFF mix was added to each sample and incubated at 65°C for 15min. Thereafter, 4µl of RNA sample buffer was added and 20µl were loaded on a RNA gel. Gels were run for in 2l 1xFGRB for 4h at 75V. For RNA transfer to nylon membranes (Amersham Hybond-N) the blotting sandwich was assembled in the following order: 3x whatman paper, gel, membrane, 3x whatman paper, paper towels, glass plate and weight bottle. Air bubbles were gently removed with an eprouvette and the membrane was enframed with laboratory film. All whatman papers were wet in 20x SSC and finally the blotting apparatus was filled up with 20x SSC on both sides.

Next day, membranes were cross linked in an UV incubation chamber followed by incubation in 10% acetic acid and transfer to 5% acetic acid with methylene blue (0,02g per 100ml). Membranes were washed in dH2O and stained ribobands were photographed. The destained membranes were transferred to glass bottles and shaken for 3h at 65°C in the hybridization oven. During prehybridization probes were p32 labelled (Stratagene Prime-It II random primer labelling kit) by mixing 7,5µl water, 10µl DNA template and 7µl random primer 9mer on ice. After 5min incubation at 95°C and short centrifugation, 7µl 5x dATP mix, 1µl Klenow polymerase and 2,5µl p32 α- dATP (in the isotope lab) was added to the samples. All DNA templates were created by PCR

from genomic DNA. After incubation on 37°C for 1h the samples were centrifuged, 60µl of sigma-TE was added and loaded on a self made sephadex column (place small whatman paper at the bottom of a 1ml syringe, fill up with sephadex and centrifuge 22sec 2200rpm in a 15ml tube) and centrifuge for 22sec at 2200rpm. The labelled probes were transferred to 1,5ml tubes, labelled properly and used for hybridization or stored at -20°C. After denaturation at 95°C for 5min, a maximum volume of 20µl probe was added to the hybridization buffer and incubated at 65°C over night. Next day membranes were incubated in washing buffer for 2x 15min at room temperature, 2x15min at 65°C, placed into plastic bags and incubated in a phosphoimager cassette over night (for fast results place a film on the membranes, incubate at -80C° and develope after 3h.

Nucleosome Scanning Assay (NuSA)

Over night cultured cells were diluted to OD600=0,25 and grown to logarithmic phase (OD=1-1,2). 45ml cells were treated with 0,4M NaCl and cross linked with 1,4ml 37% formaldehyde (1% final concentration) for 20min at room temperature. Crosslink was stopped by adding 2,5ml 2,5M glycine (125mM final concentration) for 5min. Cells were centrifuged for 2min at 2200rpm and washed twice with cold TBS buffer. Pellets were resuspended in 8ml buffer Z2, shaken on 30°C for 15min and spheroplasts were centrifuged for 10min at 3000rpm (4°C). Pellets were frozen at -20°C after removing the supernatant. Pellets were resuspended in 1,5ml NPS buffer and divided into 3x600µl in 1,5ml tubes. One series was stored at -20°C, two series were digested with 1,5µl Mnase (15U/µl) at 37°C for 45 and 50min, respectively. Digestions were incubated on ice and 12µl 500mM EDTA was added to stop the reaction. Reversal of crosslink was performed by adding 60µl of 10% SDS, 10µl of 10mg/ml proteinase K (Merck) and incubation at 65°C over night.

DNA was either frozen at -20°C or extracted with 2x equal volume phenol and 1x equal volume chloroform. DNA was precipitated with 20µl of 5M NaCl and 600µl isopropanol at -20°C for 30min and centrifuged for 30min at 14000rpm (4°C). Dry pellets were resuspended in 40µl of TE buffer, 2µl of 10mg/ml RNAse A was added and incubated at 37°C for 1h. Thereafter, 19µl of 5M LiCl was added at 4°C for 20min and centrifuged for 10min at 3000rpm (4°C). Supernatants were transferred into new 1,5ml tubes and DNA was precipitated with 10µl 3M NaAc and 150µl EtOH for 10min at room

temperature. Dry pellets were resuspended in 20µl TE and loaded on a 1% agarose gel. Monosomal bands (100-200bp) were cut out of the gel and DNA was purified by gel extraction (Kit). DNA was eluted in 30µl elution buffer and 3µl per sample was loaded on a 1% agarose gel. DNA amount of samples were adjusted for quantitative PCR.

Chromatin Immunopreciptiation (ChIP)

Over night cultured cells were grown from OD=0,1 to 0,5. Treatment of cells started with the latest timepoint by pouring 30ml cells into 50ml cap tubes and adding 3,7ml of 5M NaCl (0,4M final). After the last timepoint all tubes were opened and 1,4ml formaldehyde was added, inverted and incubated for 15min at room temperature to perform cross link of DNA and proteins. The reaction was stopped by adding 2,5ml 2,5M glycine for 10min. Thereafter, all tubes were centrifuged for 2min at 2200rpm and the pellets were washed three times with cold TBS. The washed pellets were transferred into spin cap tubes, the remaining liquid was completely removed and 600µl lysis buffer and glass beads were added. Cells were broken by fast prep (4x17sec, 5min pause after 2 runs). Cell suspension was transferred to 1,5ml tubes by melting a small hole into the bottom (with a flamed needle) of the screw cap tubes and centrifugation into 1,5ml tubes (put bottom of screw cap tube into 1,5ml tube, put both tubes into a 50ml tubes and perform centrifugation 2min 2200rpm). All samples were sonicated 4x25s, centrifuged 10min and the supernatant was divided into two series: 280µl were frozen, 280µl were used for IP and 2x10µl samples were taken as inputs. Beads (Invitrogen Dynabeads Pan Mouse IgG) were washed three times with BSA/PBS (5mg/ml). After the liquid was completely removed, the beads were resuspended in the proper volume required for 30µl per sample. 30µl beads were added to each sample and incubated on a wheel over night at 4°C.

Quantitative PCR (qPCR)

ChIP DNA samples were diluted 1:6 and standards were prepared from input DNA (1:10, 1:40, 1:160, 1:640). For each primer pair, 5µl of standards and sample DNA were loaded in triplets on 96well qRT PCR plates (Eppendorf) on ice. Thereon, 20µl Master

Mix was added into each well, the plates were sealed with microseals B film (Bio-Rad) and qPCR was performed with a Mastercycler realplex ep gradient (Eppendorf).

DNA samples for NuSA were checked on a gel (2µl) and diluted depending to the intensity of the mononucleosomal band (load 2µl on agarose gel). The dilutions are usually between 1:20 and 1:500. The standard primer (*VCX1*) and a primer of the +1 nucleosome should be tested In the first qPCR run. In case that *VCX1 values* are not approximately the same in all samples, change the dilutions of the samples.

Southern Blot

Concentration of genomic DNA was checked by Nano Drop. 15-17µg genomic DNA was digested over night. The digest was loaded on a 1% agarose gel without EtBr, and run at 70V for 3-4h. Afterwards, the gel was incubated in buffer DB (1,5M NaCl, 0,5N NaOH) for 2x20min and buffer DN (1,5M NaCl, 1M Tris pH7,4) for 2x20min. Transfer of the DNA to nylon membranes was performed according to the Northern Blot protocol (10xSSC). Membranes were washed with 2xSSC, and UV-crosslinked (as recommended for DNA/Southern hybridization). Prehybridisation, probe labelling and incubation were performed according to the Northern Blot protocol. The following washing steps were performed:

Room temperature, 15min each
3x (2xSSC, 0,1%SDS)
65°C 15min each
3x (1xSSC 0,1%SDS)
3x (0,5xSSC 0,1%SDS)
3x (0,1xSSC 0,2%SDS)

Buffers and media

40% glucose:

40g glucose ad 100ml dH2O autoclave

YEP medium:

10g Yeast Extract, 20g Peptone ad 1000ml, autoclave

YEPD medium:

add 2% Glucose to YEP medium

YEPD plates:

10g Yeast Extract, 20g Peptone, 2% Glucose, 20g Agar ad 1000ml, autoclave

SC medium:

6,7g Yeast Nitrogen Base (w/o amino acids), 2% Glucose, 10ml 100x aminoacid mix (w/o Ura, His, Leu, Trp), 20g Agar ad 1000ml, autoclave, add Ura, His, Leu, Trp before use.

SC plates:

see SC medium +25g Agar ad 1000ml, autoclave

Plasmid transformation buffer (*S.cerevisiae*):

0,2M LiAc, 40% PEG 3350, 100mM dTT, filter sterilize

Northern Blot:

RNA extraction buffer:

50mM Tris pH7-7,4, 130mM NaCl, 5mM EDTA, 5% SDS, filter sterilize

DEPC water:

1ml DEPC ad 1000ml dH2O, autoclave

RNA sample buffer:

See DNA sample buffer, use DEPC water

5xFGRB:

50ml 1M MOPS, 40ml 0,5M NaAc pH7, 5ml 0,5M EDTA, 405ml dH2O, filter sterilize

FFF Mix:

200µl 5xFGRB, 350µl formaldehyde, 1000µl formamide

RNA gel:

Boil 4,08g Agarose in 223ml DEPC water, dissolve Agarose and add 72ml 5x FGRB and 64,8ml formaldehyde.

20x SSC:

Dissolve 175,3g NaCl and 88,2g NaCl in 1 litre dH_2O and adjust pH to 7 with HCl.

Sigma- TE:

10mM Tris, 1mM EDTA in RNAse-free water (Sigma)

Hybridization buffer:

0,5M sodium phosphate buffer (19,3g/l NaH2PO4, 64g/l Na2HPO4), 7%SDS, 1mM EDTA pH=8

Washing buffer:

0,5x SSC, 0,1% SDS

Nucleosome Scanning Assay:

Buffer Z2:

1M Sorbitol, 50mM Tris/Cl pH=7,4, 10mM ß-Mercaptoethanol

Zymolyase solution:

10mg/ml zymolyase in 40% Glycerol and 60% Buffer Z

Buffer NPS:

0,5mM Spermidine, 0,075% NP40, 50mM NaCl, 10mM Tris/Cl pH=7,4, 5mM $MgCl_2$, 1mM $CaCl_2$, 1mM ß-Mercaptoethanol (add fresh)

MNase buffer:

10mM Tris/Cl pH=7,5, 10mM NaCl, 100µg/ml BSA

Chromatin Immunoprecipitation:

ChIP lysis buffer:

50mM Hepes, 1M NaCl, 0,1M EDTA pH8, 1% Triton X100, 1µg/ml Sodium Deoxycholate, 1mM PMSF (add fresh), Complete Protease Inhibitor (add fresh), 40µg/ml Benzamidine (add fresh).

ChIP Wash Buffer:

10mM Tris/Cl pH=8, 250mM LiCl, 1mM EDTA pH=8, 0,5% NP-40, 5mg/ml Sodium Deoxycholate

6. References

Adkins, M. W., S. R. Howar and J. K. Tyler (2004). Chromatin disassembly mediated by the histone chaperone Asf1 is essential for transcriptional activation of the yeast *PHO5* and *PHO8* genes. Molecular cell. **14:** 657-666.

Adkins, M. W., S. K. Williams, J. Linger and J. K. Tyler (2007). "Chromatin disassembly from the *PHO5* promoter is essential for the recruitment of the general transcription machinery and coactivators." Molecular and Cellular biology **27**(18): 6372-6382.

Alejandro-Osorio, A. L., D. J. Huebert, D. T. Porcaro, M. E. Sonntag, S. Nillasithanukroh, J. L. Will and A. P. Gasch (2009). "The histone deacetylase Rpd3p is required for transient changes in genomic expression in response to stress." Genome Biology **10**(5): R57.

Alepuz, P. M., E. de Nadal, M. Zapater, G. Ammerer and F. Posas (2003). "Osmostress-induced transcription by Hot1 depends on a Hog1-mediated recruitment of the RNA Pol II." The EMBO journal **22**(10): 2433-2442.

Alepuz, P. M., A. Jovanovic, V. Reiser and G. Ammerer (2001). "Stress-induced map kinase Hog1 is part of transcription activation complexes." Molecular Cell **7**(4): 767-777.

Almer, A. and W. Horz (1986). "Nuclease hypersensitive regions with adjacent positioned nucleosomes mark the gene boundaries of the *PHO5*/*PHO3* locus in yeast." The EMBO journal **5**(10): 2681-2687.

Altaf, M., A. Auger, J. Monnet-Saksouk, J. Brodeur, S. Piquet, M. Cramet, N. Bouchard, N. Lacoste, R. T. Utley, L. Gaudreau and J. Cote (2010). "NuA4-dependent acetylation of nucleosomal histones H4 and H2A directly stimulates incorporation of H2A.Z by the SWR1 complex." The Journal of Biological Chemistry **285**(21): 15966-15977.

Bai, L., A. Ondracka and F. R. Cross (2011). "Multiple sequence-specific factors generate the nucleosome-depleted region on CLN2 promoter." Molecular Cell **42**(4): 465-476.

Bao, Y. and X. Shen (2007). "INO80 subfamily of chromatin remodeling complexes." Mutation Research **618**(1-2): 18-29.

Bao, Y. and X. Shen (2011). "SnapShot: Chromatin remodeling: INO80 and SWR1." Cell **144**(1): 158-158 e152.

Barbaric, S., T. Luckenbach, A. Schmid, D. Blaschke, W. Horz and P. Korber (2007). "Redundancy of chromatin remodeling pathways for the induction of the yeast *PHO5* promoter in vivo." The Journal of Biological Chemistry **282**(38): 27610-27621.

Bhaumik, S. R. and M. R. Green (2001). "SAGA is an essential in vivo target of the yeast acidic activator Gal4p." Genes & Development **15**(15): 1935-1945.

Bryant, G. O., V. Prabhu, M. Floer, X. Wang, D. Spagna, D. Schreiber and M. Ptashne (2008). "Activator control of nucleosome occupancy in activation and repression of transcription." PLoS Biology **6**(12): 2928-2939.

Bryant, G. O. and M. Ptashne (2003). "Independent recruitment in vivo by Gal4 of two complexes required for transcription." Molecular Cell **11**(5): 1301-1309.

Cairns, B. R., H. Erdjument-Bromage, P. Tempst, F. Winston and R. D. Kornberg (1998). "Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF." Molecular Cell **2**(5): 639-651.

Carrozza, M. J., B. Li, L. Florens, T. Suganuma, S. K. Swanson, K. K. Lee, W. J. Shia, S. Anderson, J. Yates, M. P. Washburn and J. L. Workman (2005). "Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription." Cell **123**(4): 581-592.

Chandy, M., J. L. Gutierrez, P. Prochasson and J. L. Workman (2006). "SWI/SNF displaces SAGA-acetylated nucleosomes." Eukaryotic Cell **5**(10): 1738-1747.

Chechik, G. and D. Koller (2009). "Timing of gene expression responses to environmental changes." Journal of Computational Biology : a Journal of Computational Molecular Cell Biology **16**(2): 279-290.

De Nadal, E., M. Zapater, P. M. Alepuz, L. Sumoy, G. Mas and F. Posas (2004). "The MAPK Hog1 recruits Rpd3 histone deacetylase to activate osmoresponsive genes." Nature **427**(6972): 370-374.

Ebbert, R., A. Birkmann and H. J. Schüller (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." Molecular Microbiology **32**(4): 741-751.

Erkina, T. Y., Y. Zou, S. Freeling, V. I. Vorobyev and A. M. Erkine (2010). "Functional interplay between chromatin remodeling complexes RSC, SWI/SNF and ISWI in regulation of yeast heat shock genes." Nucleic Acids Research **38**(5): 1441-1449.

Fenn, S., D. Breitsprecher, C. B. Gerhold, G. Witte, J. Faix and K. P. Hopfner (2011). "Structural biochemistry of nuclear actin-related proteins 4 and 8 reveals their interaction with actin." The EMBO journal **30**(11): 2153-2166.

Ferreiro, I., M. Barragan, A. Gubern, E. Ballestar, M. Joaquin and F. Posas (2010). "The p38 SAPK is recruited to chromatin via its interaction with transcription factors." The Journal of Biological Chemistry **285**(41): 31819-31828.

Floer, M., X. Wang, V. Prabhu, G. Berrozpe, S. Narayan, D. Spagna, D. Alvarez, J. Kendall, A. Krasnitz, A. Stepansky, J. Hicks, G. O. Bryant and M. Ptashne (2010). "A RSC/nucleosome complex determines chromatin architecture and facilitates activator binding." Cell **141**(3): 407-418.

Ford, J., O. Odeyale, A. Eskandar, N. Kouba and C. H. Shen (2007). "A SWI/SNF- and INO80-dependent nucleosome movement at the *INO1* promoter." Biochemical and Biophysical Research Communications **361**(4): 974-979.

Ford, J., O. Odeyale and C. H. Shen (2008). "Activator-dependent recruitment of SWI/SNF and INO80 during *INO1* activation." Biochemical and Biophysical Research Communications **373**(4): 602-606.

Görner, W., E. Durchschlag, M. T. Martinez-Pastor, F. Estruch, G. Ammerer, B. Hamilton, H. Ruis and C. Schüller (1998). "Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity." Genes & Development **12**(4): 586-597.

Görzer, I., C. Schüller, E. Heidenreich, L. Krupanska, K. Kuchler and U. Wintersberger (2003). "The nuclear actin-related protein Act3p/Arp4p of Saccharomyces cerevisiae is involved in transcription regulation of stress genes." Molecular Microbiology **50**(4): 1155-1171.

Gregory, P. D., A. Schmid, M. Zavari, M. Munsterkotter and W. Horz (1999). "Chromatin remodelling at the *PHO8* promoter requires SWI-SNF and SAGA at a step subsequent to activator binding." The EMBO journal **18**(22): 6407-6414.

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

Haruki, H., J. Nishikawa and U. K. Laemmli (2008). "The anchor-away technique: rapid, conditional establishment of yeast mutant phenotypes." Molecular Cell **31**(6): 925-932.

Hassan, A. H., K. E. Neely and J. L. Workman (2001). "Histone acetyltransferase complexes stabilize swi/snf binding to promoter nucleosomes." Cell **104**(6): 817-827.

Hassan, A. H., P. Prochasson, K. E. Neely, S. C. Galasinski, M. Chandy, M. J. Carrozza and J. L. Workman (2002). "Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes." Cell **111**(3): 369-379.

Hirsch, J. P. and S. A. Henry (1986). "Expression of the *Saccharomyces cerevisiae* inositol-1-phosphate synthase (*INO1*) gene is regulated by factors that affect phospholipid synthesis." Molecular and Cellular Biology **6**(10): 3320-3328.

Ivanovska, I., P. E. Jacques, O. J. Rando, F. Robert and F. Winston (2011). "Control of chromatin structure by spt6: different consequences in coding and regulatory regions." Molecular and Cellular Biology **31**(3): 531-541.

Jiang, C. and B. F. Pugh (2009). "Nucleosome positioning and gene regulation: advances through genomics." Nature Reviews. Genetics **10**(3): 161-172.

Kaplan, C. D., L. Laprade and F. Winston (2003). "Transcription elongation factors repress transcription initiation from cryptic sites." Science **301**(5636): 1096-1099.

Kashiwaba, S., K. Kitahashi, T. Watanabe, F. Onoda, M. Ohtsu and Y. Murakami (2010). "The mammalian INO80 complex is recruited to DNA damage sites in an ARP8 dependent manner." Biochemical and Biophysical Research Communications **402**(4): 619-625.

Kast, D. J. and R. Dominguez (2011). "Arp you ready for actin in the nucleus?" The EMBO journal **30**(11): 2097-2098.

Keogh, M. C., S. K. Kurdistani, S. A. Morris, S. H. Ahn, V. Podolny, S. R. Collins, M. Schuldiner, K. Chin, T. Punna, N. J. Thompson, C. Boone, A. Emili, J. S. Weissman, T. R. Hughes, B. D. Strahl, M. Grunstein, J. F. Greenblatt, S. Buratowski and N. J. Krogan (2005). "Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex." Cell **123**(4): 593-605.

Keogh, M. C., T. A. Mennella, C. Sawa, S. Berthelet, N. J. Krogan, A. Wolek, V. Podolny, L. R. Carpenter, J. F. Greenblatt, K. Baetz and S. Buratowski (2006). "The *Saccharomyces cerevisiae* histone H2A variant Htz1 is acetylated by NuA4." Genes & Development **20**(6): 660-665.

Klopf, E., L. Paskova, C. Sole, G. Mas, A. Petryshyn, F. Posas, U. Wintersberger, G. Ammerer and C. Schüller (2009). "Cooperation between the INO80 complex and histone chaperones determines adaptation of stress gene transcription in the yeast *Saccharomyces cerevisiae*." Molecular and Cellular Biology **29**(18): 4994-5007.

Korber, P., S. Barbaric, T. Luckenbach, A. Schmid, U. J. Schermer, D. Blaschke and W. Horz (2006). "The histone chaperone Asf1 increases the rate of histone eviction at the yeast *PHO5* and *PHO8* promoters." The Journal of Biological Chemistry **281**(9): 5539-5545.

Korber, P., T. Luckenbach, D. Blaschke and W. Horz (2004). "Evidence for histone eviction in trans upon induction of the yeast *PHO5* promoter." Molecular and Cellular Biology **24**(24): 10965-10974.

Kuo, M. H., J. E. Brownell, R. E. Sobel, T. A. Ranalli, R. G. Cook, D. G. Edmondson, S. Y. Roth and C. D. Allis (1996). "Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines." Nature **383**(6597): 269-272.

Larschan, E. and F. Winston (2001). "The *S. cerevisiae* SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4." Genes & Development **15**(15): 1946-1956.

Lindstrom, K. C., J. C. Vary, Jr., M. R. Parthun, J. Delrow and T. Tsukiyama (2006). "Isw1 functions in parallel with the NuA4 and Swr1 complexes in stress-induced gene repression." Molecular and Cellular Biology **26**(16): 6117-6129.

Liu, C. L., T. Kaplan, M. Kim, S. Buratowski, S. L. Schreiber, N. Friedman and O. J. Rando (2005). "Single-nucleosome mapping of histone modifications in S. cerevisiae." PLoS biology **3**(10): e328.

Luger, K., A. W. Mader, R. K. Richmond, D. F. Sargent and T. J. Richmond (1997). "Crystal structure of the nucleosome core particle at 2.8 A resolution." Nature **389**(6648): 251-260.

Mason, P. B. and K. Struhl (2003). "The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo." Molecular and Cellular Biology **23**(22): 8323-8333.

Mizuguchi, G., X. Shen, J. Landry, W. H. Wu, S. Sen and C. Wu (2004). "ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex." Science **303**(5656): 343-348.

Morrison, A. J., J. Highland, N. J. Krogan, A. Arbel-Eden, J. F. Greenblatt, J. E. Haber and X. Shen (2004). "INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair." Cell **119**(6): 767-775.

Niederacher, G., E. Klopf and C. Schüller (2011). "Interplay of dynamic transcription and chromatin remodeling: lessons from yeast." International Journal of Molecular Sciences **12**(8): 4758-4769.

Papamichos-Chronakis, M., S. Watanabe, O. J. Rando and C. L. Peterson (2011). "Global regulation of H2A.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity." Cell **144**(2): 200-213.

Raisner, R. M., P. D. Hartley, M. D. Meneghini, M. Z. Bao, C. L. Liu, S. L. Schreiber, O. J. Rando and H. D. Madhani (2005). "Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin." Cell **123**(2): 233-248.

Rougvie, A. E. and J. T. Lis (1988). "The RNA polymerase II molecule at the 5' end of the uninduced *hsp70* gene of D. melanogaster is transcriptionally engaged." Cell **54**(6): 795-804.

Ruiz-Roig, C., C. Vieitez, F. Posas and E. de Nadal (2010). "The Rpd3L HDAC complex is essential for the heat stress response in yeast." Molecular Microbiology **76**(4): 1049-1062.

Rundlett, S. E., A. A. Carmen, N. Suka, B. M. Turner and M. Grunstein (1998). "Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3." Nature **392**(6678): 831-835.

Santisteban, M. S., M. Hang and M. M. Smith (2011). "Histone Variant H2A.Z and RNA Polymerase II Transcription Elongation." Molecular and Cellular biology.

Schmid, A., K. D. Fascher and W. Horz (1992). "Nucleosome disruption at the yeast *PHO5* promoter upon *PHO5* induction occurs in the absence of DNA replication." Cell **71**(5): 853-864.

Schwabish, M. A. and K. Struhl (2006). "Asf1 mediates histone eviction and deposition during elongation by RNA polymerase II." Molecular Cell **22**(3): 415-422.

Shen, X., G. Mizuguchi, A. Hamiche and C. Wu (2000). "A chromatin remodelling complex involved in transcription and DNA processing." Nature **406**(6795): 541-544.

Shen, X., R. Ranallo, E. Choi and C. Wu (2003). "Involvement of actin-related proteins in ATP-dependent chromatin remodeling." Molecular Cell **12**(1): 147-155.

Szerlong, H., K. Hinata, R. Viswanathan, H. Erdjument-Bromage, P. Tempst and B. R. Cairns (2008). "The HSA domain binds nuclear actin-related proteins to regulate chromatin-remodeling ATPases." Nature Structural & Molecular Biology **15**(5): 469-476.

Traven, A., B. Jelicic and M. Sopta (2006). "Yeast Gal4: a transcriptional paradigm revisited." EMBO reports **7**(5): 496-499.

Yoh, S. M., H. Cho, L. Pickle, R. M. Evans and K. A. Jones (2007). "The Spt6 SH2 domain binds Ser2-P RNAPII to direct Iws1-dependent mRNA splicing and export." Genes & Development **21**(2): 160-174.

Yosef, N. and A. Regev (2011). "Impulse control: temporal dynamics in gene transcription." Cell **144**(6): 886-896.

Yuan, G. C., Y. J. Liu, M. F. Dion, M. D. Slack, L. F. Wu, S. J. Altschuler and O. J. Rando (2005). "Genome-scale identification of nucleosome positions in *S. cerevisiae*." Science **309**(5734): 626-630.

Zhang, H., D. N. Roberts and B. R. Cairns (2005). "Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss." Cell **123**(2): 219-231.

7. Appendix

7.1 Abstract

Eukaryotic cells activate stress genes in response to environmental changes, such as high temperature or salt concentration. The expression of these genes is a highly regulated process and dependent on a variety of different factors. In the nucleus, DNA is wrapped around histone octamers forming nucleosomes which are the basic packaging units of chromatin. Euchromatin represents a less condensed, transcriptionally active form of chromatin, whereas heterochromatin is densely packaged and transcriptionally inactive. Tight compaction of DNA decreases accessibility for the transcriptional machinery and therefore reduces expression of the affected genes. Several factors change the chromatin structure and thereby influence gene expression. In this work I studied the ATP dependent chromatin remodeling complex INO80 in the simple eukaryote *S.cerevisiae* to examine the role of INO80 during active transcription of stress genes. The INO80 complex is assembled of 15 subunits and exhibits a genome wide role for repression of strongly induced genes. In combination with earlier observations my data suggest that a module of nuclear Actin, Arp4 and Arp8 as well as Arp5 and the Ino80 ATPase are the key components of INO80 mediated transcriptional repression at stress genes. Mutants lacking one of these subunits show hyperinduced and prolonged transcription of stress genes. Furthermore, ATP hydrolysis by the Ino80 ATPase domain is required for INO80 mediated inhibition of stress genes. After induction of transcription, promoter nucleosomes are completely depleted most probably by the activity of the transcriptional machinery and INO80 is involved in their reconstitution. In order to understand the molecular role of the complex during the transcription cycle I tried to identify the subunits that are required for recruitment of the INO80 complex to stress genes. My data indicate that the Actin related Proteins (Arps) are not required for targeting of the complex to sites of active transcription. Hence, Arps are important for activity of the complex at stress genes.

7.2 Zusammenfassung

Eukaryotische Zellen aktivieren sogenannte Stressgene und reagieren damit auf plötzliche Veränderungen in ihrer Umwelt, wie z.B. einer Erhöhung der Temperatur oder Osmolarität. Die Expression dieser Gene ist ein stark regulierter Prozess der unter anderem vom Verpackungsgrad der DNA abhängig ist. DNA liegt im Zellkern in der komprimierten Form des Chromatins vor, dessen kleinste Grundeinheit das sogenannte Nukleosom darstellt. Nukleosomen bestehen aus 8 Untereinheiten von Histon-Proteinen, um die 147bp der DNA gewickelt sind. Das Ausmaß der Komprimierung von bestimmten DNA Abschnitten bestimmt deren Zugänglichkeit für die transkriptionelle Maschinerie und somit ob bestimmte Gene exprimiert werden. Es gibt verschiedene Proteine und Proteinkomplexe innerhalb des Zellkerns, die Chromatinstrukturen verändern können. In dieser Arbeit beschäftige ich mich mit dem aus insgesamt 15 Untereinheiten aufgebauten, ATP abhängigen Chromatin Remodeling Complex INO80. Der einfache Eukaryont *S.cerevisiae* dient als Modellsystem um den Einfluss dieses Proteinkomplexes auf die transkriptionelle Regulation von Stressgenen zu untersuchen. Ein Modul aus Actin, Arp4 und Arp8, sowie Arp5 und die Ino80 ATPase sind jene Hauptakteure des INO80 Komplexes, die einen inhibierenden Effekt auf die Transkription von Stressgenen ausüben. In Mutanten welchen eine dieser Untereinheiten fehlt, wird eine verstärkte, länger andauernde Transkription von Stressgenen beobachtet. Dieser Effekt scheint ein allgemeiner Mechanismus zur Regulierung sehr stark exprimierter Gene zu sein. Der inhibierende Effekt des INO80 Komplex beruht sehr wahrscheinlich auf der Wiederherstellung jener Nukleosomen, die durch transkriptionelle Aktivierung vom Promoter entfernt werden. Die Wiederherstellung der Promoternukleosomen ist in Mutanten, welchen entweder Arp8 oder Ino80 fehlt, zeitlich stark verzögert. Mithilfe einer ATPase- inaktiven Mutante der Ino80 Untereinheit wird gezeigt, dass ATP Hydrolyse für die transkriptionell inhibierende Wirkung des INO80 Komplex notwendig ist. Außerdem wurde versucht Untereinheiten zu identifizieren, die für die Rekrutierung des Komplexes zu Stressgenen notwendig sind. Nach den vorliegenden Ergebnissen werden Arp4, Arp5 und Arp8 nicht für diese Aufgabe benötigt und sind somit hauptsächlich für die Aktivität des INO80 Komplexes zuständig.

7.3 Curriculum Vitae

Gerhard Niederacher

Spengergasse 53/6a, A 1050 Vienna

Personal Data

E-Mail	gerhard.niederacher@univie.ac.at
Date of Birth	05.05.1984
Place of Birth	Zell am See, Austria
Nationality	Austria

Education

2010-2012	Diploma Work (Univ.Doz. Dr. Christoph Schüller)
2005-2012	Molecular Biology, University of Vienna
2004-2005	Human Medicine, University of Vienna
2003	Military Service
9.6.2002	Matura
1994-2002	Bundesrealgymnasium Zell am See
1990-1994	Elementary School Zell am See

Practical trainings

02.2010- 10.2011	Diploma Work at MFPL, Department of Biochemistry and Cell Biology, University of Vienna and UFT Tulln, DAGZ, University of Natural Resources and Life Sciences Vienna, Univ. Doz. Dr. Christoph Schüller.
08-09.2009	AKH Wien, KIMCL, Medical University of Vienna, O.Univ.Prof. Dr. Oswald Wagner
04-05.2009	MFPL, Department of Immunobiology, University of Vienna, Ao.Univ.Prof. Pavel Kovarik
02.2009	MFPL, Department of Biochemistry and Cell biology, University of Vienna, Univ. Doz. Dr. Christoph Schüller
08.2008	AKH Wien, Nephrology, Medical University of Vienna, Dr. Markus Wahrmann

Articles	Niederacher G, Klopf E, Schüller C. (2011) Interplay of dynamic transcription and chromatin remodeling: lessons from yeast. Int J Mol Sci. PMID: 21954323
Languages	German, English