Deciphering the role of Aft1p in chromosome stability

by

Akil Hamza

Department of Biochemistry, Microbiology, and Immunology

Submitted in partial fulfillment

of the requirements for the degree of

Master of Science

Faculty of Graduate Studies

The University of Ottawa

Ottawa, ON

THE UNIVERSITY OF OTTAWA

ABSTRACT

The *Saccharomyces cerevisiae* iron-responsive transcription factor, Aft1p, has a well established role in regulating iron homeostasis through the transcriptional induction of iron-regulon genes. However, recent studies have implicated Aft1p in other cellular processes independent of iron-regulation such as chromosome stability. In addition, chromosome spreads and two-hybrid data suggest that Aft1p interacts with and co-localizes with kinetochore proteins, however the cellular implications of this have not been established. Here, we demonstrate that Aft1p associates with the kinetochore complex through Iml3p. Furthermore, we show that Aft1p, like Iml3p, is required for the increased association of cohesin with the pericentromere and that *aft1Δ* cells display sister chromatid cohesion defects in both mitosis and meiosis. Our work defines a new role for Aft1p in the sister chromatid cohesion pathway.

ACKNOWLEDGEMENTS

The completion of this work would not have been possible without the guidance and help of several individuals who supported me throughout my graduate studies.

I would like to thank my supervisor Dr. Kristin Baetz for her guidance, support, and patience. She has my deepest gratitude.

Past and current lab members Leslie Mitchell, Jane Usher, Mike Kennedy, Sylvain Huard, Roya Pourhanifeh, Mojgan Siahbazi, Mike Cotrut, Kristy Shulist, Jessica Desjardins, Aya Helal, Ted Sewell, Kate Boehm, Jeremy Chitpin, and Jennifer Takuski for their help, advice, scientific support, and the many good times. It was a pleasure working with all.

Dr. Adele Marston from the University of Edinburgh for providing me with the strains to complete this work, Dr. Alexandre Blais for his advice on qPCR, and Dr. Adam Rudner for the scientific help whenever I needed.

Family and friends for their unconditional love and support during my highs and lows.

And my loving wife for her continuous encouragement, love, patience and kind words.

Table of Contents:

List of Abbreviations:

APC/C	Anaphase-promoting Complex/Cyclosome
ATP	Adenosine Triphosphate
BPS	Bathophenantroline-disulphonate
CEN	Centromere
ChIP	Chromatin Immunoprecipitation
CDE	Centromere DNA Element
CoIP	Co-immunoprecipitation
CT	Cycle Threshold
CTF	Chromosome Transmission Fidelity
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetraacetic Acid
EGTA	Ethylene Glycol Tetraacetic Acid
GFP	Green Fluorescent Protein
HU	Hydroxyurea
IgG	Immunoglobulin
IRE	Iron-Responsive Element
ISCs	Iron-sulfur Clusters
MET	Methionine
mRNA	Messenger Ribonucleic Acid
mtDNA	Mitochondrial DNA
NLS	Nuclear Localization Signal
OD_{600}	Optical Density at 600nm
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
SDL	Synthetic Dosage Lethality
SDS-PAGE	Sodium-Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis
SGA	Synthetic Genetic Array
SL	Synthetic Lethality
TAE	Tris-acetate-EDTA
TAP	Tandem Affinity Purification
WCE	Whole Cell Extract
YPD	Yeast Peptone Dextrose

List of Figures:

List of Tables:

Chapter 1: INTRODUCTION

The mechanisms that govern chromosome stability play essential functions in cell biology. This is highlighted by the fact that gain or loss of chromosomes (aneuploidy) has been implicated in most cancers (Thompson and Compton, 2011). An important aspect of chromosome stability are the cohesive forces that hold sister chromatids together till the onset of chromosome segregation. This pathway is regulated by a highly conserved multi-subunit complex called cohesin initially discovered in the budding yeast model organism (Guacci et al., 1997; Michaelis et al., 1997). Members of the cohesin complex and the mechanisms that regulate the cohesion pathway are conserved from budding yeast to humans (Peters et al., 2008). After the discovery of cohesin, many diseases have been implicated to be linked to mutations of cohesin subunits and have been collectively termed cohesinopathies (Musio and Krantz, 2010). These include Cornelia de Lange syndrome and Roberts/SC phocomelia syndrome that are both characterized by growth defects, various developmental abnormalities and mental retardation (Gerkes et al., 2010; Jahnke et al., 2008). As such, studies that elucidate cohesion regulatory mechanisms will be very helpful in the characterization of human diseases. In this study, I am utilizing the budding yeast to characterize the role of the iron-responsive transcription factor, Aft1p, in chromosome stability and more specifically the cohesion pathway.

Aft1p and Aft2p are iron-responsive transcription factors in budding yeast

Iron is an essential nutrient required by organisms for numerous cellular processes (Kaplan et al., 2006). Iron uptake and utilization is controlled by a regulatory network that takes into account iron bioavailability (scarce amounts to extreme abundance) and cellular homeostasis (iron deficiency to iron toxicity). In the budding yeast, *Saccharomyces cerevisiae*, cellular iron levels are regulated by the transcription factors Aft1p and Aft2p (for

"activator of ferrous transport") (reviewed in (Philpott and Protchenko, 2008; Rutherford and Bird, 2004)). Iron depletion activates Aft1p (also known as Rcs1p) and Aft2p to induce the expression of approximately 25 genes referred to as the 'iron-regulon' by binding the consensus sequence 5'-PyPuCACCCPu-3' present in iron-responsive elements (IREs) of these target genes (Rutherford et al., 2003; Yamaguchi-Iwai et al., 1996). This subset of genes is involved in the uptake, compartmentalization, and utilization of iron with the end result of increasing iron levels and remodeling cellular metabolism to survive low iron conditions. Although Aft1p and Aft2p have overlapping but non-redundant roles in the transcriptional regulation of the iron-regulon (Blaiseau et al., 2001), Aft1p appears to have the more prominent role as the transcriptional effects of Aft2p are less apparent unless Aft1p is deleted (Rutherford et al., 2003). Consistent with this observation, *aft1Δ* mutant cells exhibit low ferrous iron uptake and poor growth under low-iron conditions (Casas et al., 1997; Yamaguchi-Iwai et al., 1995) while *aft2Δ* mutant cells display no phenotype under similar conditions (Courel et al., 2005; Rutherford et al., 2003). However, an *aft1Δaft2Δ* double mutant is more sensitive to low-iron growth than an *aft1Δ* mutant demonstrating the functional similarity of these two proteins and the ability of Aft2p to regulate transcription of the iron-regulon (Blaiseau et al., 2001; Rutherford et al., 2001). While Aft1p and Aft2p activate transcription of an overlapping set of target genes, there are some iron-regulon genes that are targeted solely by Aft2p including *SMF3* and *MRS4*; genes involved in vacuolar and mitochondrial iron transport, respectively (Courel et al., 2005). Furthermore, given that *aft1Δ* and *aft2Δ* mutants display distinct phenotypes and have differing genetic profiles suggests that some cellular functions are not shared between these two transcription factors (Berthelet et al., 2010).

Cellular localization and activation of Aft1p

AFT1 encodes a protein of 690 amino acids that is constitutively expressed, and under normal iron conditions, is routinely shuttled between the nucleus and the cytoplasm (Ueta et al., 2003). Nuclear export of Aft1p from the nucleus is promoted in the presence of iron and is mediated by two regions, amino acids 147-270 and 304-498 (Ueta et al., 2007). Nuclear import of Aft1p is independent of cellular iron levels and is mediated by two nuclear localization signals (NLS) at amino acids 198-225 and 332-365 as well as a direct interaction with the nuclear import factor Pse1p (Ueta et al., 2003). Other important domains of Aft1p include the N-terminal region which is believed to act as a DNA binding domain and the C-terminal region which functions as the transcriptional activation domain (Yamaguchi-Iwai et al., 1995; Yamaguchi-Iwai et al., 2002).

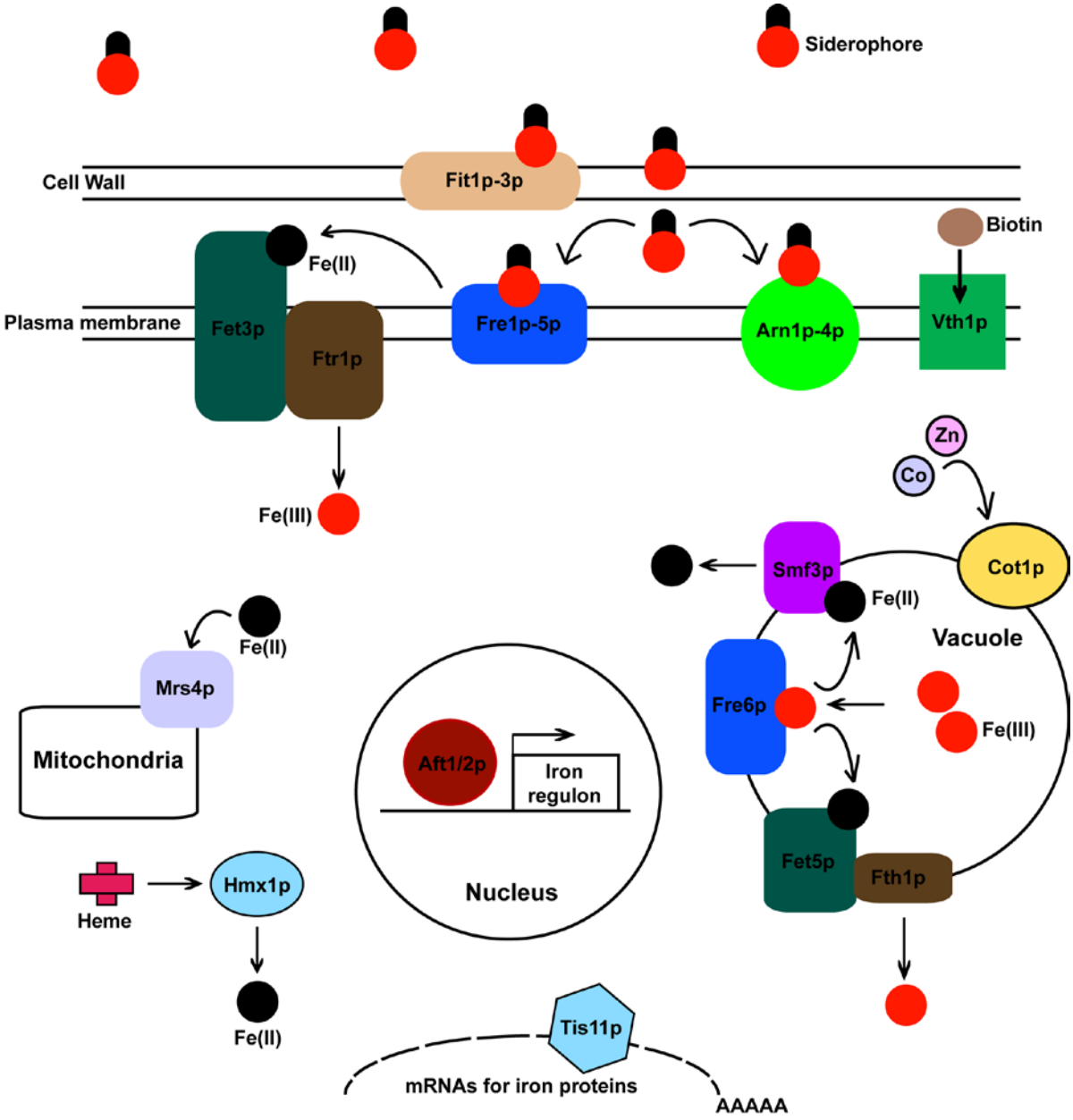
In contrast to some metalloregulatory transcriptional activators in yeast that respond directly to metal binding (such as those involved in zinc and copper homeostasis), Aft1p is not regulated through the direct binding of iron (Rutherford and Bird, 2004). Instead, Aft1p is activated in response to mitochondrial iron-sulfur (Fe-S) cluster (ISCs) biogenesis as opposed to cytosolic iron levels (Li et al., 2011). Consistent with this, defects in mitochondrial Fe-S biogenesis lead to iron accumulation in the mitochondria and constitutive induction of the iron-regulon while bearing no effect on cytosolic iron levels (Chen et al., 2004; Foury and Talibi, 2001; Lange et al., 2000); while loss of mitochondrial DNA (mtDNA) resulting in decreased levels of iron-sulfur clusters induces the iron-regulon (Veatch et al., 2009).

The 'iron-regulon'

Iron-regulon genes encode proteins that increase cellular iron levels through acquisition of iron from the environment, mobilization of stored iron in the vacuole and

mitochondria, and the remodeling of metabolic pathways that favor iron-independent over iron-dependent processes (Figure 1) (reviewed in (Philpott and Protchenko, 2008; Rutherford and Bird, 2004)). The majority of the iron-regulon genes encode proteins involved in uptake and acquisition of free iron at the cell surface. As iron in the environment is found in the ferric form (Fe^{3+}), ferric reductases (*FRE1, FRE2, FRE3, FRE4, FRE5*) reduce ferric iron to ferrous iron (Fe^{2+}) as a substrate for the high-affinity transport system comprising the integral membrane ferroxidase (*FET3*) and the iron permease (*FTR1*). Since copper is required for the proper functioning of Fet3p, genes involved in trafficking (*ATX1*) and transport of copper (*CCC2*) are also regulated as part of the iron-regulon. Additionally, iron from the environment can be acquired from low molecular weight organic molecules called siderophores that chelate iron with high affinity and specificity. Although it cannot synthesize its own siderophores, the budding yeast is able to utilize the iron bound to siderophores produced by other microorganisms such as fungi and bacteria. This is mediated through siderophore transporters (*ARN1, ARN2, ARN3, ARN4*) and cell wall mannoproteins (*FIT1, FIT2, FIT3*) that facilitate iron uptake through retention of iron-bound siderophores at the cell wall by an unknown mechanism. Other iron-regulon genes function through the mobilization of stored iron in the cell. Although some iron is present in the cytosol, mitochondria and other compartments, the main iron storage compartment is the vacuole. As such, iron-depletion induces iron-regulon genes (*FRE6*, *SMF3*, *FET5*, *FTH1*, *COT1*) that function in the efflux of iron from the vacuole to the cytosol. Like other members of the FRE family, *FRE6* encodes a ferric reductase that functions exclusively at the vacuolar membrane. Smf3p is a metal transporter that functions with Fre6p to move iron out of the vacuole, while Fet5p and Fth1p are thought to form a vacuolar iron transporter complex that mimics Fet3p and Ftr1p at the cell surface. To buffer the toxic effects of other metals in iron-

Figure 1: Overview of cellular response to iron-deprivation in *Saccharomyces cerevisiae*. (As adapted from Philpott and Protchenko, 2008)


Siderophore
Cell Wall
Fit1p-3p
Biotin
Fe(II)
Plasma membrane
Fet3p
Ftr1p
Fre1p-5p
Arn1p-4p
Vth1p
Fe(III)
Zn
Co
Smf3p
Cot1p
Fe(II)
Vacuole
Mrs4p
Fe(II)
Mitochondria
Fre6p
Fe(III)
Aft1/2p
Iron
regulon
Nucleus
Fet5p
Fth1p
Hmx1p
Heme
Fe(II)
Tis11p
mRNAs for iron proteins
AAAAA

deficient cells such as copper, zinc, cobalt, and manganese, the iron-regulon gene (*COT1*) encodes a vacuolar transporter that sequesters these metals in the vacuole. Other iron-regulon genes function at the mitochondria as heme-degrading enzymes (*HMX1*) and in iron transport (*MRS4*). In addition to increasing cellular iron levels through acquisition of new iron from the environment and mobilization of stored iron from the vacuole and mitochondria, some iron-regulon genes encode proteins that alter metabolic pathways in order to conserve iron usage. These include an mRNA binding protein (*TIS11*) that destabilizes transcripts that encode enzymes utilizing iron as a cofactor, and a high affinity biotin transporter (*VTH1*) that is up-regulated to allow the cell to bypass the iron-dependent biotin biosynthesis pathway.

Aft1p has been implicated in other cellular functions

Although Aft1p has an established role as an iron-responsive transcription factor, many studies have linked Aft1p to other cellular roles including chromosome stability, cell cycle regulation, DNA damage, cell wall stability, and mitochondrial function. *AFT1* over-expression results in the transcriptional modulation of more than 200 genes implicated in a variety of cellular processes many of which are not transcriptionally induced under iron-limited conditions (Shakoury-Elizeh et al., 2004); while microarray studies investigating the transcriptional effects of Aft1p in iron-replete media showed that deletion of *AFT1* resulted in the up-regulation of 239 genes and the down-regulation of 350 genes (Pagani et al., 2007). However, genome-wide chromatin immunoprecipitation (ChIP) arrays or ChIP-on-ChIP studies have not successfully mapped Aft1p chromosomal locations (Harbison et al., 2004; Lee et al., 2002); therefore it is not known how many of the Aft1p-induced genes are directly regulated by Aft1p (through binding to target sequences in their promoters) or result from downstream transcriptional cascades. Furthermore, evidence for a role for Aft1p in cell cycle

regulation came from studies showing that over-expression of *AFT1* results in G1 arrest due to the iron-dependent inhibition of translation of G1 cyclins by an undetermined mechanism (Casas et al., 1997; Philpott et al., 1998); while *aft1Δ* mutant cells are significantly larger than wild-type cells suggesting a delay of these mutant cells in G1 phase (Jorgensen et al., 2002; White et al., 2009). Additionally, *aft1Δ* mutants display hypersensitivity to hydroxyurea (HU) that can be suppressed by exogenous iron (Dubacq et al., 2006). Hydroxyurea is an inhibitor of ribonucleotide reductase and DNA replication, suggesting an iron-dependent role for Aft1p in cell cycle regulation and HU response. Moreover, chemical genomics studies indicated a possible role for Aft1p in DNA damage response as it was found that *aft1Δ* mutants are hypersensitive to inter-strand cross-linking DNA-damaging agents such as carboplatin and cisplatin (Lee et al., 2005); while cisplatin treatment activates Aft1p to induce the iron-regulon (Kimura et al., 2007). Even though *aft2Δ* and other mutants of the iron-regulon were not found to be sensitive to any DNA-damaging agents (Lee et al., 2005), the hypersensitivity of *aft1Δ* mutant cells to cisplatin was suppressed by addition of exogenous iron (Berthelet et al., 2010). Overall, this suggests that Aft1p has the dominant role in iron regulation and plays an important iron-dependent role in DNA damage response as many enzymes and proteins involved in DNA repair utilize iron as a cofactor (Lill and Muhlenhoff, 2008). Additional studies exploiting synthetic genetic array (SGA) techniques have also implicated Aft1p in numerous cellular processes (Berthelet et al., 2010; Measday et al., 2005). Synthetic genetic array is a methodology that exploits the yeast gene deletion and over-expression collection to systematically identify genetic interactions (Tong and Boone, 2006). Our lab carried out complementary genome-wide synthetic lethality (SL) and synthetic dosage lethality (SDL) screens and identified 77 deletion mutants that cannot

tolerate deletion or over-expression of *AFT1* under normal iron conditions (Berthelet et al., 2010). As expected, the genetic interaction network indentified genes implicated in cell cycle and DNA damage repair as well as further predicting possible roles for Aft1p in cell-wall assembly, protein transport, and mitochondrial function.

Aft1p functions in chromosome stability

While many of these pathways linked to Aft1p function can be attributed to perturbations in iron homoeostasis, the link between Aft1p and chromosome stability has been shown to be iron-independent (Berthelet et al., 2010). Evidence implicating Aft1p in chromosome stability first originated from synthetic genetic array (SGA) studies that determined *aft1Δ* mutants could not tolerate either over-expression or loss-of-function of kinetochore genes under normal iron conditions (Berthelet et al., 2010; Measday et al., 2005). Additionally, a genome-wide study identified *aft1Δ* mutants as having mild chromosome segregation defects during meiosis indicative of cohesion defects (Marston et al., 2004). Moreover, chromosome transmission fidelity (CTF) assays determined that *aft1Δ* mutant cells have defects in maintaining artificial chromosome fragments (Berthelet et al., 2010; Measday et al., 2005; Yuen et al., 2007). Recently, our lab has shown that *aft1Δ* mutant cells are hypersensitive to benomyl treatment as compared to wild-type cells (Berthelet et al., 2010). Benomyl is a microtubule-destabilizing drug and many mutants with defects in chromosome stability share the same sensitivity (Sora et al., 1982). In contrast to most chemical sensitivities and phenotypes of *aft1Δ* cells, neither the benomyl sensitivity nor chromosome transmission fidelity defects of *aft1Δ* cells can be rescued by increased extracellular iron in media (Berthelet et al., 2010). Furthermore, *AFT2* and other iron-regulon genes were not identified in the genome-wide studies that implicated Aft1p in chromosome stability; while *aft2Δ* mutant cells did not display any sensitivity to benomyl treatment

(Berthelet et al., 2010). Overall, these results suggest that the role of Aft1p in chromosome stability is not related to regulation of the iron-regulon or a role in iron homeostasis.

It is not known whether Aft1p's contribution to chromosome stability is dependent on transcription. Our lab explored the possibility that Aft1p may be regulating the transcription of key genes required for benomyl resistance and chromosome stability by employing microarray studies comparing the transcriptional response of *aft1Δ* cells versus wild-type cells grown in iron-replete media (Berthelet et al., 2010). Of the genes identified whose transcription is decreased two-fold or more, only deletion mutants of *CTF19* have been demonstrated to be both hypersensitive to benomyl and display chromosome transmission defects (Hyland et al., 1999). Ctf19p is a member of the COMA complex of the kinetochore proteins that is required for accurate chromosome segregation (Pot et al., 2003). To further explore the possibility that Ctf19p levels may be responsible for the benomyl sensitivity of *aft1Δ* cells, our lab showed that neither an extra genomic clone of *CTF19* nor an HA-tagged *CTF19* fusion clone could suppress the benomyl sensitivity of *aft1Δ* cells (Appendix A). Furthermore, I carried out a modified chromatin-immunoprecipitation (mChIP) experiment using TAP-tagged Aft1p to determine whether I can detect Aft1p at the promoter of *CTF19*. My results demonstrate that while I was able to amplify the positive control *FET3*, I saw no enrichment of the *CTF19* promoter possibly suggesting that Aft1p indirectly regulates the transcription of *CTF19*. While these results indicate that the benomyl sensitivity of *aft1Δ* cells is not due to the transcriptional regulation of *CTF19*, the possibility that some of Aft1p's role in chromosome stability is mediated through transcription cannot be eliminated.

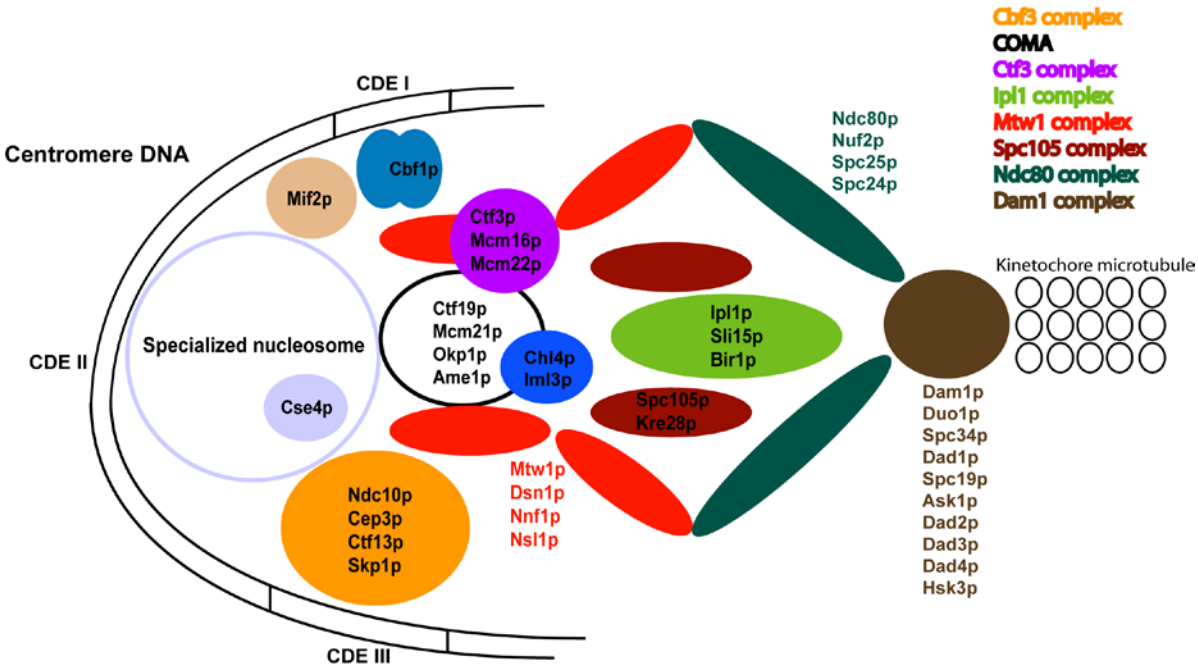
Aft1p localizes to the kinetochore

Many lines of evidence suggest that Aft1p has a role at the yeast kinetochore. The kinetochore is a key component for proper chromosome segregation and is composed of

essential and non-essential subunits organized in a hierarchical assembly of multi-protein sub-complexes (Figure 2) (reviewed in (McAinsh et al., 2003; Westermann et al., 2007)). This protein complex assembles on the centromere and mediates the attachment and movement of chromosomes along the microtubules of the mitotic spindle. The budding yeast centromere is approximately 125bp long and is composed of three regions termed CDEI, CDEII, and CDEIII (centromere DNA element) wrapped around a specialized nucleosome containing a histone H3-variant called Cse4p (CENP-A in humans) (reviewed in (Roy and Sanyal, 2011; Verdaasdonk and Bloom, 2011). The kinetochore proteins are grouped into three general categories based on their relative position between centromeric DNA and the microtubules. The inner kinetochore proteins assemble directly on centromeric DNA and are essential for DNA looping and recruitment of other kinetochore proteins. The inner kinetochore proteins include Cbf1p which binds the 8bp CDEI region, the essential Cbf3 complex which is composed of 4 subunits (Ndc10p, Cep3p, Ctf13p, Skp1p) recognizing the 25bp CDEIII region, and the specialized nucleosome contacting the 76-86bp A-T rich CDEII region. The microtubule-binding proteins include the essential Dam1 complex and microtubule motor proteins required to generate chromosome movement. The central kinetochore proteins link the inner kinetochore and microtubule-binding proteins and serve different regulatory and structural functions.

Indication of an Aft1p-kinetochore interaction first originated from indirect immunofluorescence analysis on chromosome spreads which found that Aft1p co-localizes with the kinetochore protein Ndc10p (Measday et al., 2005). Moreover, Aft1p has been shown to interact with the kinetochore protein, Iml3p, by yeast-two hybrid assays (Wong et al., 2007; Yu et al., 2008). Iml3p is a central kinetochore protein and peripheral member of the Ctf19 complex and like many kinetochore proteins, *iml3Δ* mutants are hypersensitive to

Figure 2: Schematic diagram of the budding yeast kinetochore illustrating the different multi-protein sub-complexes arranged in a hierarchical organization. The kinetochore is localized to the centromere (CDE: centromere DNA element). (As adapted from Westermann et al., 2007)


Cbf3 complex
COMA
Ctf3 complex
Ipl1 complex
Mtw1 complex
Spc105 complex
Ndc80 complex
Dam1 complex
CDE I
Centromere DNA
Cbf1p
Mif2p
Ctf3p
Mcm16p
Mcm22p
Ndc80p
Nuf2p
Spc25p
Spc24p
Kinetochore microtubule
Ctf19p
Mcm21p
Okp1p
Ame1p
Chl4p
Iml3p
Ipl1p
Sli15p
Bir1p
CDE II
Specialized nucleosome
Cse4p
Spc105p
Kre28p
Dam1p
Duo1p
Spc34p
Dad1p
Spc19p
Ask1p
Dad2p
Dad3p
Dad4p
Hsk3p
Mtw1p
Dsn1p
Nnf1p
Nsl1p
Ndc10p
Cep3p
Ctf13p
Skp1p
CDE III

benomyl treatment (Pot et al., 2003). The Ctf19 complex is composed of the COMA sub-complex (Ctf19p, Okp1p, Mcm21p, Ame1p) along with Chl4p and Iml3p (De Wulf et al., 2003; Pot et al., 2003). Studies investigating the hierarchical dependencies of the kinetochore proteins have revealed that Iml3p localization to the kinetochore is dependent on Chl4p, which in turn is dependent on Ctf19p (Figure 3) (Pot et al., 2003). While very little is known about Iml3p or associated Ctf19 complex protein function, they have been shown to be required for the increased association of cohesin at the pericentromere and centromere regions in both mitosis and meiosis (Fernius and Marston, 2009; Marston et al., 2004); however, the molecular mechanism by which members of the Ctf19 complex recruit or maintain cohesin has yet to be understood.

Cohesin and sister chromatid cohesion

From the onset of DNA replication until chromosome segregation, sister chromatids are paired and physically linked by the multi-subunit cohesin complex (reviewed in (Nasmyth and Haering, 2009; Ocampo-Hafalla and Uhlmann, 2011; Oliveira and Nasmyth, 2010)). Sister chromatid cohesion counteracts the bipolar pulling forces of the microtubules thus assuring the proper bi-orientation of chromosomes and preventing their random segregation during mitosis. Mitotic cohesin is composed of at least six essential subunits (Smc1p, Smc3p, Scc1p, Scc3p, Pds5p, Wpl1p) forming a ring-like complex that embraces sister chromatids until the metaphase to anaphase transition (Figure 4). Smc1p and Smc3p (structural maintenance of chromosomes) are flexible rod shaped proteins that form a heterodimer with an ATPase domain. While very little is known about the molecular consequences of ATP hydrolysis in establishing cohesion, it appears to be essential for assembly and loading of the cohesin ring onto chromosomes. Scc1p (sister chromatid cohesion) interconnects and stabilizes the Smc1p/Smc3p interaction and recruits the other

Figure 3: Schematic diagram of the budding yeast kinetochore highlighting the hierarchical dependencies of the Ctf19 sub-complex. The kinetochore localization of Iml3p is dependent on Chl4p which in turn is dependent on Ctf19p. (As adapted from Pot et al., 2003)

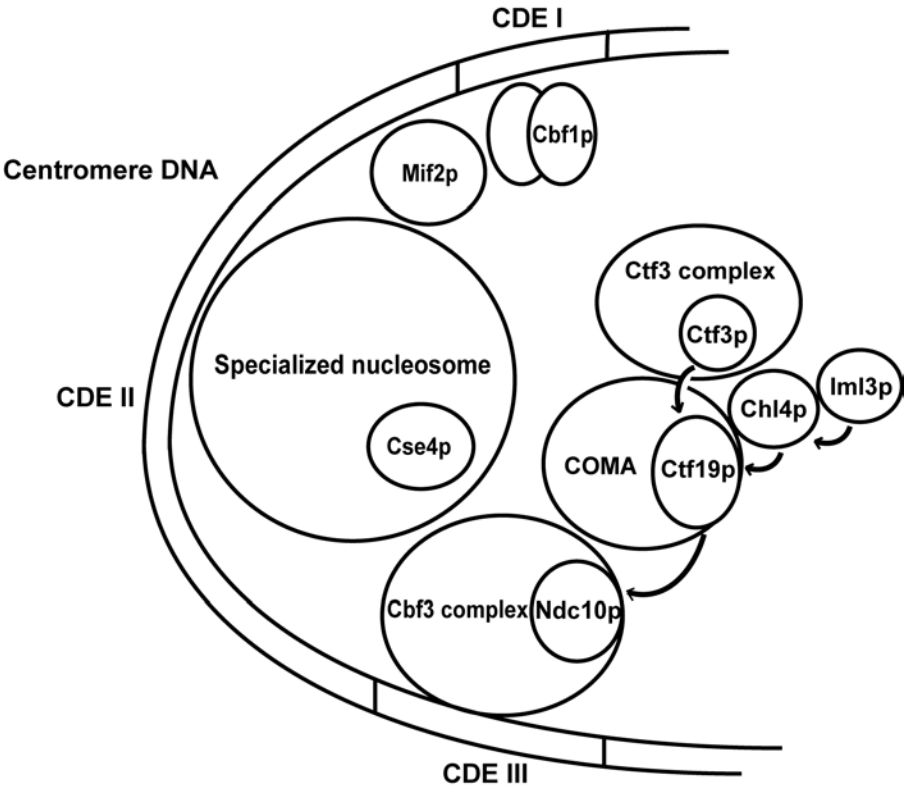
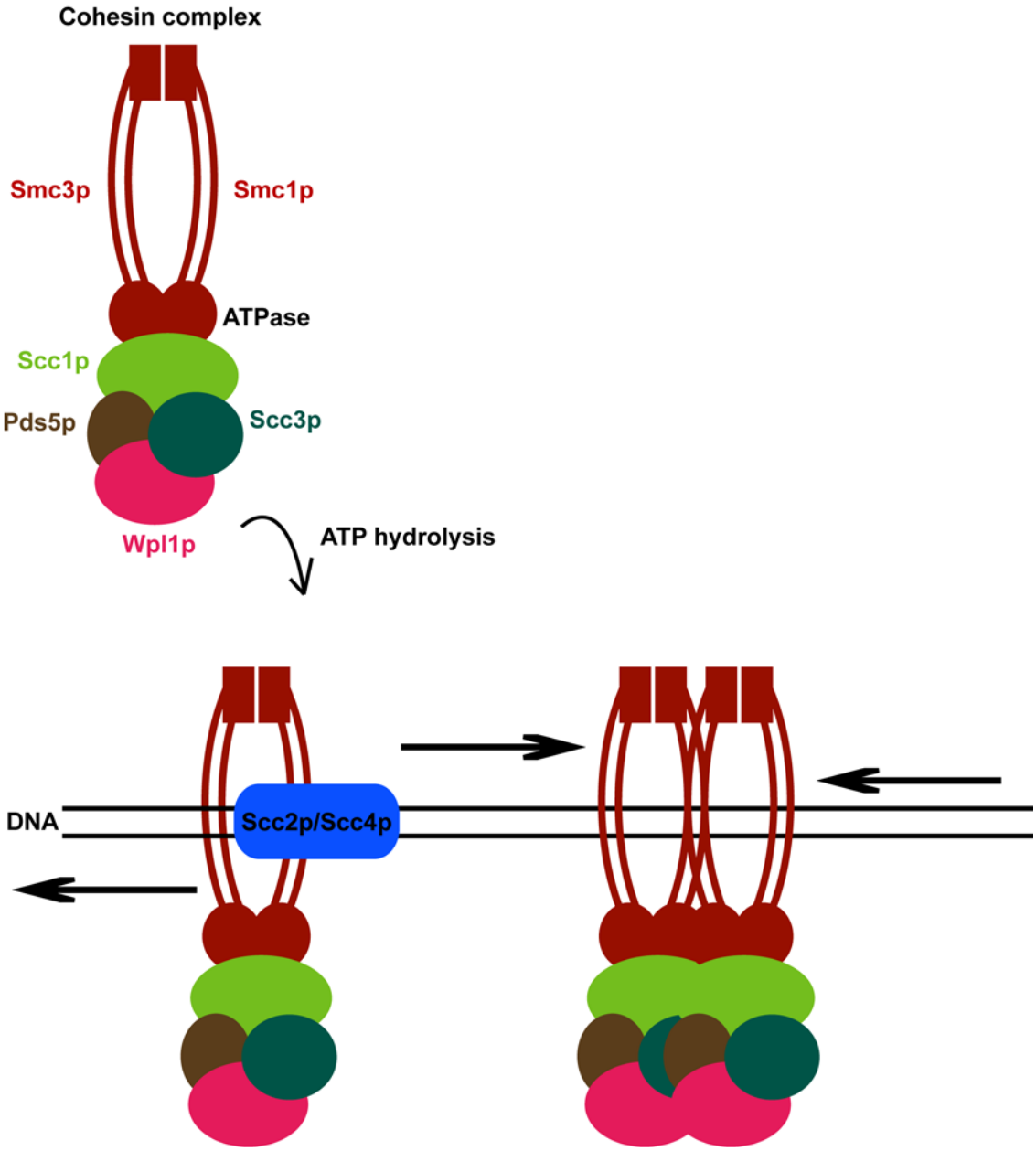

CDE I
Centromere DNA
Cbf1p
Mif2p
Ctf3 complex
Ctf3p
Specialized nucleosome
CDE II
Iml3p
Chl4p
Cse4p
COMA
Ctf19p
Cbf3 complex
Ndc10p
CDE III

Figure 4: Schematic diagram depicting subunits of the cohesin complex and model of cohesin loading and sliding along DNA. (As adapted from Ocampo-Hafalla and Uhlmann, 2011)

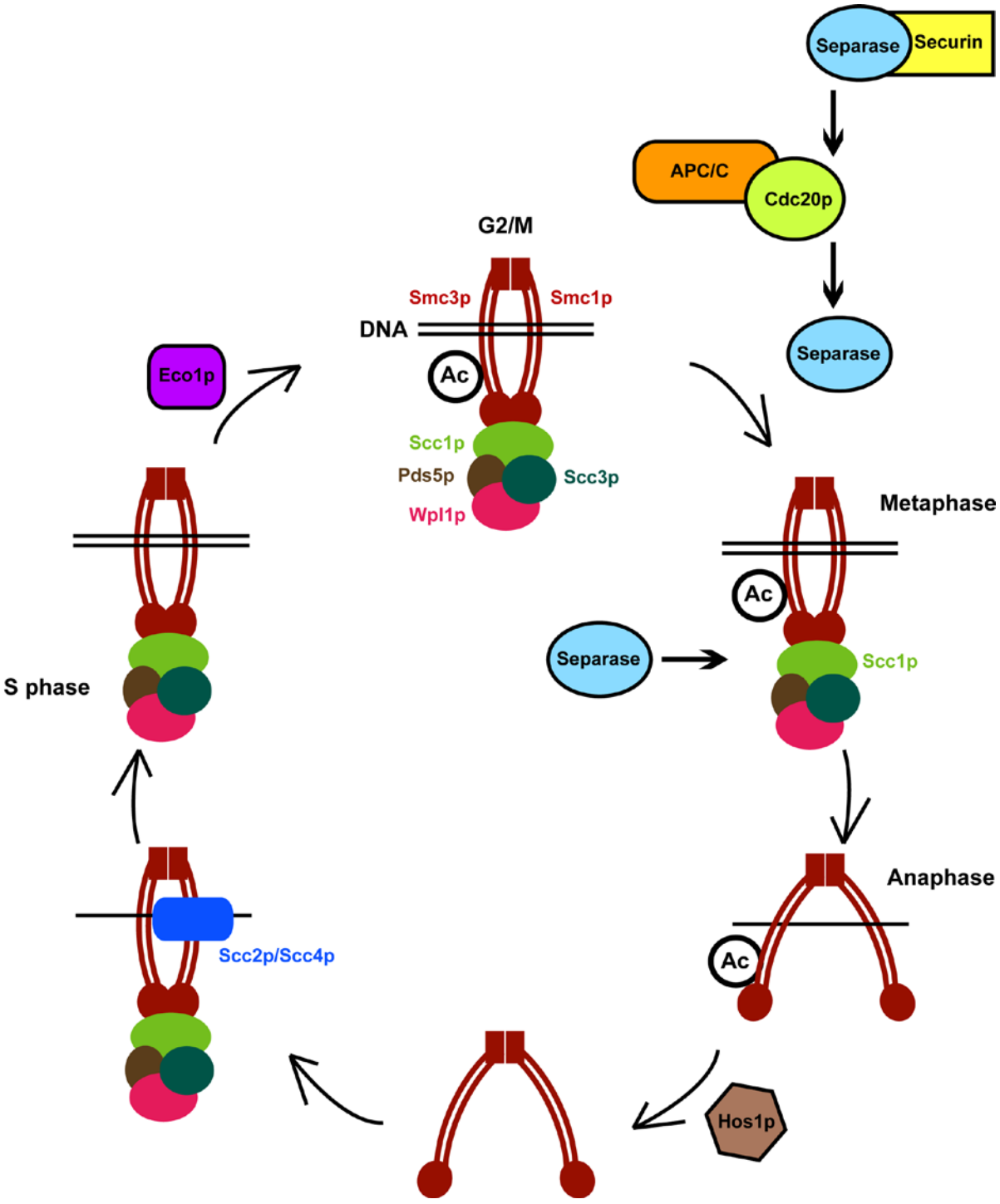

Cohesin complex
Smc3p
Smc1p
ATPase
Scc1p
Pds5p
Scc3p
Wpl1p
ATP hydrolysis
DNA
Scc2p/Scc4p

cohesin subunits. The functions of these other subunits are poorly defined but it is thought that while Scc3p is required to maintain cohesion, the Pds5p-Wpl1p complex counteracts the establishment of cohesion by an unknown mechanism (Sutani et al., 2009). A separate complex composed of the Scc2p and Scc4p proteins functions in the initial loading of cohesin but not the establishment or maintenance of cohesion.

Prior to DNA replication, cohesin is loaded onto chromosomes in a manner dependent on the Scc2p-Scc4p cohesin loader complex. Studies investigating the distribution of cohesin have found that the cohesin ring is highly enriched at the 125bp centromere and a ~50kb domain surrounding the centromere termed the pericentromere, and that this enrichment is dependent on a functional kinetochore (Weber et al., 2004). On the other hand, cohesin distribution along the chromosome arm is more dispersed (every 10-15kb) and spans shorter distances (approximately 0.8kb). Although no consensus sequence has been identified, cohesin preferentially binds A-T rich sequences and localizes to intergenic regions. The distribution of the Scc2p-Scc4p loader complex however was found to be different as it primarily localized to the promoters of actively transcribing genes (Lengronne et al., 2004). Moreover, mutations that inhibit ATP hydrolysis demonstrate the accumulation of cohesin at Scc2p-Scc4p binding sites (Hu et al., 2011). As such, the current model proposes that cohesin is initially loaded onto Scc2p-Scc4p binding sites after which it either slides or reloads to its permanent location (Ocampo-Hafalla and Uhlmann, 2011).

Once cohesin is loaded, establishment of cohesion between sister chromatids requires the acetylation of two lysine residues (K112 and K113) on Smc3p by the acetyltransferase, Eco1p (Figure 5) (reviewed in (Skibbens, 2009, 2010)). This establishes a functional linkage that is maintained until sister chromatid separation at the metaphase to anaphase transition. In a highly regulated sequence of events, the metaphase to anaphase transition is initiated

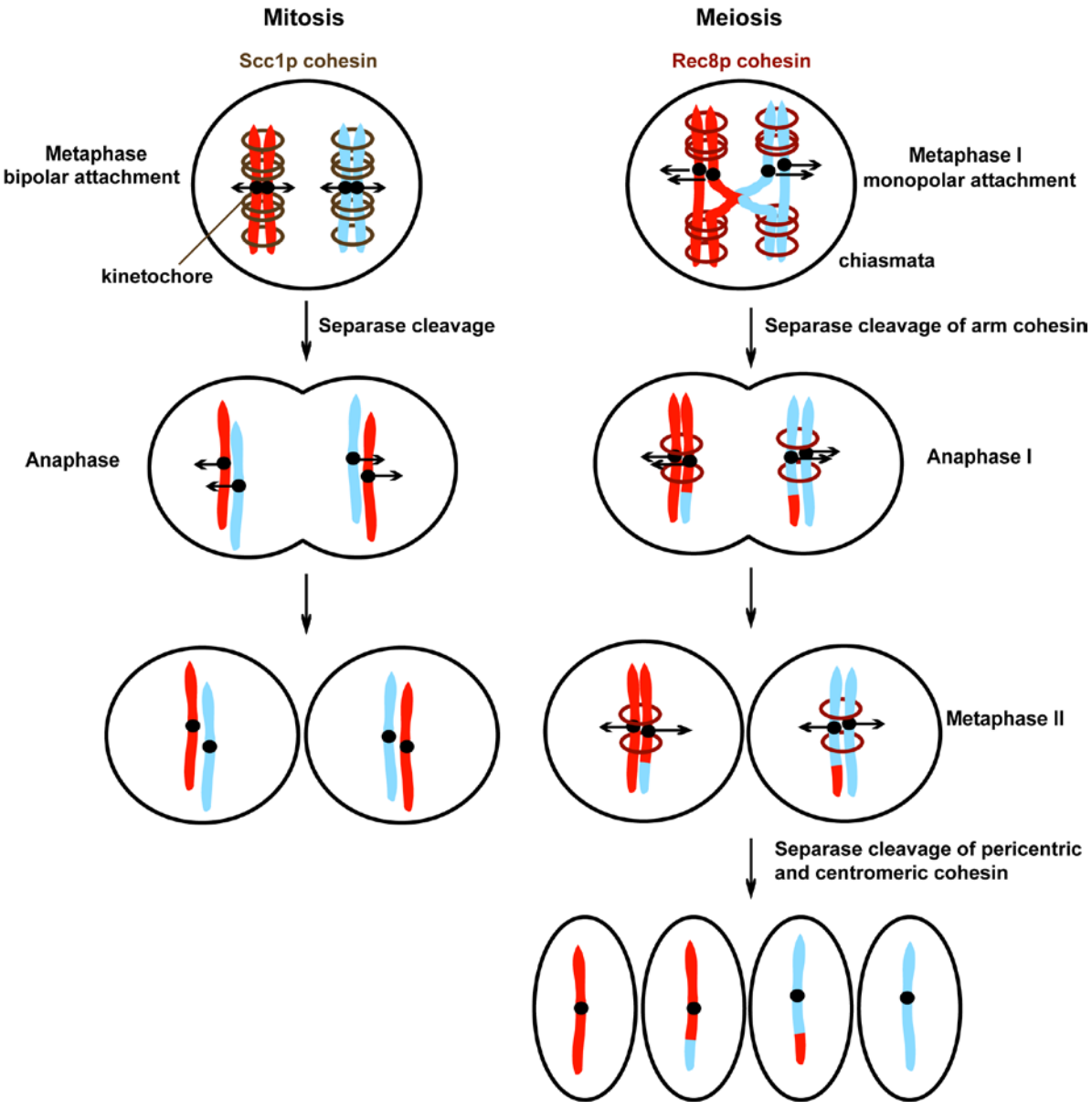
Figure 5: Schematic diagram illustrating cohesin loading and cohesion establishment throughout the cell cycle. (As adapted from Beckouet et al., 2010)


Separase
Securin
APC/C
Cdc20p
Separase
G2/M
Smc3p
Smc1p
DNA
Ac
Scc1p
Pds5p
Scc3p
Wpl1p
Eco1p
Metaphase
Ac
Separase
Scc1p
S phase
Anaphase
Ac
Scc2p/Scc4p
Hos1p

once all chromosomes biorient, thereby releasing Cdc20p from its Mad2p inhibitor. Cdc20p activates the APC/C (anaphase-promoting complex/cyclosome) which is a multi-subunit ubiquitin ligase that directs the ubiquitination and proteolysis of several proteins at the onset of anaphase. The APC/C^{Cdc20} complex recruits a separate complex composed of an inactive separase (Esp1p in budding yeast) and its inhibitor securin (Pds1p in budding yeast). The APC/C^{Cdc20} dependent ubiquitination and subsequent proteolysis of securin releases and activates separase to mediate the cleavage of Scc1p by an unknown mechanism thereby triggering chromosome separation. Following Scc1p cleavage, Smc3p is deacetylated at anaphase by the deacetylase Hos1p; a required step as cohesion establishment in the next cell cycle is dependent on de novo acetylation by Eco1p (Beckouet et al., 2010).

While most of the subunits that function in mitotic cohesion also play a role in meiotic cohesion, the major difference is that the mitotic subunit Scc1p is replaced by its meiosis-specific paralog Rec8p (Figure 6) (reviewed in (Bardhan, 2010; Sakuno and Watanabe, 2009)). In meiosis, cohesion has distinct roles as it has to account for two rounds of chromosome segregation (meiosis I and II), monopolar attachment of sister kinetochores during meiosis I, and chiasmata. In the first meiotic division (meiosis I), homologous chromosomes are paired to allow for chiasmata and it is this homologous recombination that provides tension between the chromosomes and the meiotic spindle at metaphase I. In contrast, cohesion between sister chromatids is what maintains the tension in metaphase of mitosis. The resolution of chiasmata in meiosis cannot occur unless sister chromatid cohesion is lost along the chromosomal arms while still maintained at pericentromeric locations. This step-wise reduction of cohesion is meiosis-specific and ensures the separation of homologous chromosomes during meiosis I while still maintaining the physical link between sister chromatids until anaphase II through retention of pericentromeric cohesion.

Figure 6: Schematic diagram highlighting the differences between mitotic and meiotic cohesin. (As adapted from Sakuno and Watanabe, 2009)


Mitosis
Meiosis
Scc1p cohesin
Rec8p cohesin
Metaphase
bipolar attachment
kinetochore
Metaphase I
monopolar attachment
chiasmata
Separase cleavage
Separase cleavage of arm cohesin
Anaphase
Anaphase I
Metaphase II
Separase cleavage of pericentric
and centromeric cohesin

This targeted loss of cohesion at chromosomal arms is achieved through the protection of Rec8p at the pericentromere from cleavage by separase. The protein responsible for this is shugoshin (Sgo1p in budding yeast) and it is thought that shugoshin protects pericentromeric cohesion by opposing the phosphorylation of Rec8p. Following the first meiotic division, sister chromatids become bioriented and pericentromeric cohesion is lost to allow sister chromatid separation in a manner similar to mitosis.

Iml3p and pericentromeric cohesion

Pericentromeric cohesion is important as it provides the necessary tension between sister chromatids and the microtubule pulling forces during mitosis and meiosis (Stephens et al., 2011). This is especially important in meiosis given that all arm cohesion is lost after the first meiotic division and sister chromatids are linked only via pericentric cohesion during the second meiotic division (Marston and Amon, 2004). Studies investigating chromatin structure have further shown that pericentromeric DNA is linked via intramolecular cohesion thereby allowing pericentric chromatin to form an intramolecular loop around the specialized nucleosome (Bloom et al., 2006). As a result, chromatin adopts a cruciform structure in metaphase whereby the protruding pericentric intramolecular loops of sister chromatids are oriented bi-directionally with the centromere situated at the apex of each loop. Thus, pericentric chromatin in conjunction with cohesion and other structural proteins acts as a molecular spring that balances microtubule forces (Stephens et al., 2011). This model accounts for how DNA regions with the most separation during metaphase are also the most highly enriched in cohesion.

Several studies have demonstrated a link between the Ctf19 kinetochore complex and pericentromeric cohesion. Iml3p and Chl4p were initially identified along with Sgo1p as required factors for the maintenance of pericentromeric cohesion during meiosis (Ghosh et

al., 2004; Kiburz et al., 2005; Marston et al., 2004). It was later demonstrated that all members of the Ctf19 complex and the Ctf3 complex are required for increased loading of cohesin at the pericentromere and centromere during mitosis and meiosis but not at chromosomal arm locations (Eckert et al., 2007; Fernius and Marston, 2009). Decreased levels of cohesin at the pericentromere and centromere regions resulted in increased distance and more frequent separation between sister centromeres during mitosis and chromosome segregation defects in meiosis (Fernius and Marston, 2009).

The mechanism by which Iml3p and other Ctf19 complex members promote cohesin enhancement at the centromere and pericentromere remains unknown. It is speculated that the Ctf19 complex through its Iml3p subunit directs cohesin loading at the centromere which then spreads bidirectionally throughout the pericentromere (Fernius and Marston, 2009). This model accounts for how the Ctf19 complex can localize only to centromeric regions while still impacting cohesin loading at the pericentromere. Additional support for this model comes from studies demonstrating that mitotic cohesin is detected earlier at centromeric regions than at pericentromeric sites during the cell cycle (Fernius and Marston, 2009), and that the Scc2p/Scc4p cohesin loader complex is reduced at the centromere but not at the pericentromere in *mcm21Δ* mutants (member of the COMA complex) (Ng et al., 2009). As the mechanism by which Iml3p and associated Ctf19 complex members contribute to increased cohesin binding remains elusive, identification of additional factors can help further the understanding of this pathway.

Hypothesis

Multiple studies indicate a role for the iron-responsive transcription factor, Aft1p, in chromosome stability. I hypothesize that the role of Aft1p in chromosome stability is through its interaction with the kinetochore complex.

Specific Aims

1. To identify the kinetochore protein(s) that interacts with Aft1p.

2. To elucidate the function of Aft1p in chromosome stability.

Chapter 2: MATERIALS AND METHODS

Yeast strains:

Yeast strains used in this study are listed in Table 1. Deletion mutants and epitope tag integrations made for this study were designed using a standard PCR-mediated gene insertion technique and confirmed by PCR analysis (Longtine et al., 1998).

Whole-cell extract co-immunoprecipitation (CoIP):

Cells were grown in 200mL YPD media at 30°C to mid-log phase (OD_{600} ~0.6 to 0.8) and harvested. Cell pellets were resuspended in equal volume of Tackett Extraction Buffer (20mM HEPES pH 7.4, 0.1%Tween20, 2mM $MgCl_2$, 200mM NaCl, and protease inhibitors [Sigma, catalog no. P-8215]) and an equal volume of glass beads (BioSpec Products, catalog no. 11079105). Cells were lysed through vortexing (five 1-minute blasts with incubation on ice in between vortexing) and crude whole cell extract (WCE) was separated from the beads into a new Eppendorf tube by poking a hole in the bottom of the tube using a 21-gauge needle (Becton Dickinson, catalog no. 305167) and centrifuging at 1000rpm for 1 minute. Extract was clarified at 3000rpm for 15 minutes at 4°C and whole cell extract removed. Samples were normalized by protein concentration and incubated end-over-end for 2 hours at 4°C with magnetic Dynabeads (Dynal, Invitrogen, catalog no. 143-01) crossed-linked to rabbit immunoglobulin G (IgG) (Chemicon, catalog no. PP64) or with magnetic Protein G Dynabeads (Invitrogen, catalog no. 100.03D) cross-linked to mouse HA.11 monoclonal antibody (Covance, catalog no. MMS-101P). Dynabeads were collected with a magnet, washed three times with 1mL cold Tackett Extraction Buffer and resuspended in 25µL of modified 1× loading buffer (50mM Tris, pH 6.8, 2% sodium dodecyl sulfate [SDS], 0.1% bromophenol blue, 10% glycerol). Purified and co-purified proteins were eluted from the

beads at 65°C for 10 minutes and transferred to a new tube. 2-β-mercaptoethanol was added to a final concentration of 200mM and samples were placed at 95°C for 10 minutes. Immunoprecipitates were subjected to SDS-PAGE and western blotting. Membranes were developed using Western Chemiluminescent HRP Substrate Detection System (Millipore, catalog no. WBKLS0500). Primary antibodies used are as follows: anti-TAP (Thermo Scientific, catalog no. CAB1001, 1:5000), anti-Myc (Roche, catalog no. 11667149001, 1:800), anti-HA (Covance, catalog no. MMS-101P, 1:1000). Secondary antibodies were HRP-linked, goat anti-rabbit IgG (Chemicon, catalog no. AP307P, 1:5000), and goat anti-mouse IgG (BioRad, catalog no. 170-6516, 1:5000).

ChIP/modified ChIP-multiplex PCR:

For ChIP (chromatin immunoprecipitation), cells were grown in 200mL YPD media at 30°C to mid-log phase, cross-linked, and harvested. Cells were cross-linked with 5.6mL 37% formaldehyde for 15 minutes at room temperature with occasional end-over-end rotation after which 13.6mL 2M glycine was added for 5 minutes to quench. Cell pellets were resuspended in equal volume of cold lysis buffer (100mM HEPES pH 8, 20mM Mg acetate, 300mM Na acetate, 10% glycerol, 10mM EGTA, 0.1mM EDTA, and protease inhibitors) and lysed as before. Chromatin was sheared to an average size of 500bp by sonicating 3 times 10 seconds at setting 2 (Misonix Sonicator 3000) with a 1-minute incubation on ice between each pulse. Nonident P40 [NP-40] (Roche, catalog no. 11754599001) was added to a final concentration of 1%, mixed gently for 10 minutes, and samples were centrifuged at 3000rpm for 10 minutes at 4°C. TAP-tagged proteins of interest were immunoprecipitated as mentioned previously and protein-bound beads were washed three times with 1mL cold wash buffer (lysis buffer plus 0.5% NP-40). Purified proteins

were eluted with 100µL 1X TE/1% SDS buffer at 65°C for 10 minutes and beads were washed thereafter with 150µL 1X TE/0.67% SDS buffer to obtain a final volume of 250µL. Eluates and whole cell extract samples were incubated overnight at 65°C for reverse cross-linking after which 12.5µL 20mg/mL proteinase K (Invitrogen, catalog no. 25530015) was added and samples were incubated end-over-end for 2 hours at 37°C followed by phenol/chloroform DNA extraction, ethanol precipitation in the presence of 1µg glycogen, and RNAse (Sigma, catalog no. R-5125) treatment. DNA pellets were washed with 70% ethanol, air dried, and resuspended in 50µl sterilized dH_2O. For modified ChIP (Mitchell et al., 2008), all steps are the same except there was no formaldehyde cross-linking and reverse cross-linking steps, while purified DNA was eluted with 1X TE buffer and 12.5µL 20mg/mL proteinase K at 37°C followed by DNA extraction. Immunoprecipitated DNA was amplified using multiplex-PCR with the following primer pairs: *CEN3*-F (5'GCGATCAGCGCCAAACAATATGG), *CEN3*-R (5'GAGCAAAACTTCCACCAGTAAACG) obtained from (Krogan et al., 2004); *FET3*-F (5'GGTCCCTACAGTACGCTGAG), *FET3*-R (5'GGATCGACTGTTTGAGTGCATCC); *CTF19*-F (5'CCTGGATGAAACCCACTCGAA), *CTF19*-R (5'GAGTAACTTGCACAGCTATTGG); *TEL5*-F (5′GGCTGTCAGAATATGGGGCCGTAGTA), *TEL5*-R (5'CACCCCGAAGCTGCTTTCACAATAC). PCR products were resolved on a 3% agarose gel and visualized with ethidium bromide.

ChIP-qPCR:

Cells were grown in 100mL YPD media at 25°C to mid-log phase after which benomyl (30µg/mL) (Sigma, catalog no. 38158-6) and nocodazole (15µg/mL) (Sigma, catalog no. M1404) were added for 3 hours and nocodazole (7.5µg/mL) was re-added every

hour to depolymerize microtubules. ChIP was performed as described previously. Immunoprecipitated DNA was subjected to qPCR in a 10µl EvaGreen (BioRad, catalog no. 172-5201) reaction using the BioRad MiniOpticon Real-Time PCR System. ChIP enrichment/input values were calculated as described (Fernius and Marston, 2009). Briefly, ΔCT was calculated according to the formula: $\Delta CT = (CT_{(ChIP)} - (CT_{(Input)} - \log E \text{ (Input dilution factor)}))$ where E represents the specific primer efficiency value. Enrichment/input values were obtained from the following formula: $E^{-\Delta CT}$ and final results are the average of three independent experiments. Primer sequences were obtained from (Fernius and Marston, 2009) where the pericentromere (P), centromere (C), and arm (A) primers correspond to primer pairs P2, C1, and A3 respectively. The primer sequences used are as follows: P-F (5'ATTGTTTAGAAACGGGAACA), P-R (5'GTTCAACTCTCTGCATCTCC); C-F (5'ACACGAGCCAGAAATAGTAAC), C-R (5'TGATTATAAGCATGTGACCTTT); A-F (5'GAAAGCGACCAGCTAGATTA), A-R (5'CAAACGCTTTAACACACAAG).

Dot assays and growth conditions:

Yeast strains were grown in YPD at 25°C to mid-log phase and then spotted in ten-fold serial dilutions (OD_{600}= 0.1, 0.01, 0.001, 0.0001) onto YPD plates and incubated at 25°C, 30°C and 33°C for 2 days before epi-white imaging using the Molecular Imager ChemiDoc XRS System (BioRad). Dot assay experiments were repeated in triplicate using different isolates of each strain.

Cohesion assays and microscopy:

For the mitotic cohesion assay (Fernius and Marston, 2009), cells were grown at 30°C in 50mL media lacking methionine to OD_{600}~0.5 and α-mating factor (Sigma, catalog no. T6901) was added to a final concentration of 10µg/mL for 3 hours to arrest cells in the

G_1 phase. After washing the synchronized cells three times with sterilized dH_2O to remove any residual α-factor, cells were resuspended in 50mL media supplemented with 8mM methionine and grown for 120 minutes at 30°C to arrest cells at the metaphase/anaphase transition. Cells were then fixed (Klein et al., 1999) by addition of 1/10 volume 34% formaldehyde for 10 minutes, washed once with 96% ethanol, and resuspended in 500µL KPO_4/sorbitol solution (0.1M KPO_4, 1.2M sorbitol). A total of 200 cells were scored per replicate and three replicates were performed for each sample. All cells in a field were scored and GFP dots were scored as separated if two dots were clearly visible in the same cell. If GFP dots were visible in mother and daughter cells, then these cells were not scored as that indicated cells did not arrest at the metaphase/anaphase transition and have progressed through the cell cycle. However, this was never more than 10% and was comparable in all strains. For the meiotic cohesion assay (Marston et al., 2003), diploid strains were grown for 24 hours at 30°C in YPD media to saturation then diluted to an OD_{600} ~0.2 to 0.3 into YPA media (1% yeast extract, 2% bactopeptone, 1% potassium acetate) and grown overnight to OD_{600}~1.8. Diploid cells were then resuspended in equal volume of sporulation media (0.3% potassium acetate pH 7, 0.02% raffinose) and grown for two days before fixation of cells (as described previously). Results are the mean of three replicates in which 100 tetra-nucleate cells were scored for GFP segregation patterns. Microscopy was performed on a Leica DMI6000B florescent microscope (Leica Microsystems GmbH, Wetzler Germany), equipped with a Sutter DG4 light source (Sutter Instruments, California, USA), Ludl emission filter wheel with Chroma band pass emission filters (Ludl Electronic Products Ltd., NY, USA) and Hamamatsu Orca AG camera (Hamamatsu Photonics, Herrsching am Ammersee, Germany). Images were collected and analyzed using Velocity 4.3.2 Build 23

(Perkin Elmer). Analysis was performed on images collapsed into two dimensions using the "extended focus" in Velocity.

Chapter 3: RESULTS

Aft1p interacts with the kinetochore protein Iml3p.

In order to elucidate the function of Aft1p in chromosome stability, I first sought to identify the individual kinetochore protein or protein sub-complex that interacts with Aft1p. As Iml3p was found to interact with Aft1p by yeast-two hybrid (Wong et al., 2007; Yu et al., 2008), I decided to confirm this physical interaction by a secondary method. Co-immunoprecipitation experiments with strains containing TAP-tagged Aft1p and Myc-tagged Iml3p, Chl4p, or Ctf19p confirmed that Aft1p-TAP can co-purify proteins of the Ctf19 sub-complex (Figure 7A, 8A, and 9A lanes 4). To further dissect the Aft1p-kinetochore interaction, I systematically created deletion mutant strains based on the established hierarchical dependencies of the Ctf19 sub-complex.

I first investigated whether the Aft1p-Ctf19p interaction (Figure 7A, lane 4) is dependent on Chl4p or Iml3p. In a *chl4Δ* background that abolishes the Iml3p-Ctf19p interaction, the Aft1p-Ctf19p interaction was disrupted (Figure 7A, lane 5) indicating that this interaction was either through Chl4p or Iml3p. In an *iml3Δ* background that maintains the Chl4p-Ctf19p interaction, albeit in a reduced fashion (Fernius and Marston, 2009; Pot et al., 2003), the Aft1p-Ctf19p interaction was also disrupted (Figure 7A, lane 6). These results suggest that Aft1p is co-purifying Chl4p and Ctf19p through Iml3p. To confirm this I next examined the effect of *ctf19Δ* and *iml3Δ* mutations on the Aft1p-Chl4p interaction (Figure 8A, lane 4). In a *ctf19Δ* background that maintains the Iml3p-Chl4p complex (Pot et al., 2003), the Aft1p-Chl4p interaction was preserved (Figure 8A, lane 5) indicating that Aft1p interacts with either Chl4p or Iml3p but not Ctf19p. In an *iml3Δ* background that maintains the Chl4p-Ctf19p interaction (Fernius and Marston, 2009; Pot et al., 2003), Aft1p could not co-immunoprecipitate Chl4p anymore (Figure 8A, lane 6) indicating that this interaction was

mediated by Iml3p. My last experiment looked directly at the consequence of *ctf19Δ* and *chl4Δ* mutations on the Aft1p-Iml3p interaction (Figure 9A, lane 4). My results confirmed the findings of the previous two experiments as I show that in both *ctf19Δ* and *chl4Δ* mutant backgrounds (Figure 9A, lanes 5 and 6) Aft1p can still co-immunoprecipitate Iml3p. Therefore, my results confirmed the Aft1p-Iml3p two hybrid interaction and indicated that Aft1p is interacting with kinetochore COMA complex subunits through Iml3p.

Figure 7: Aft1p interaction with the kinetochore protein Ctf19p is dependent on Chl4p and Iml3p. (A) Anti-TAP immunoprecipitations were performed with strains containing different combinations of TAP-tagged Aft1p and Myc-tagged Ctf19p in the presence or absence of Chl4p and Iml3p. 10% of the eluate was probed for anti-TAP while 90% of the eluate was probed for anti-Myc. **(B)** Schematic representation illustrating the Aft1p-Ctf19p interaction in the presence or absence of Chl4p and Iml3p. (IP: immunoprecipitations; WCE: whole-cell extract)

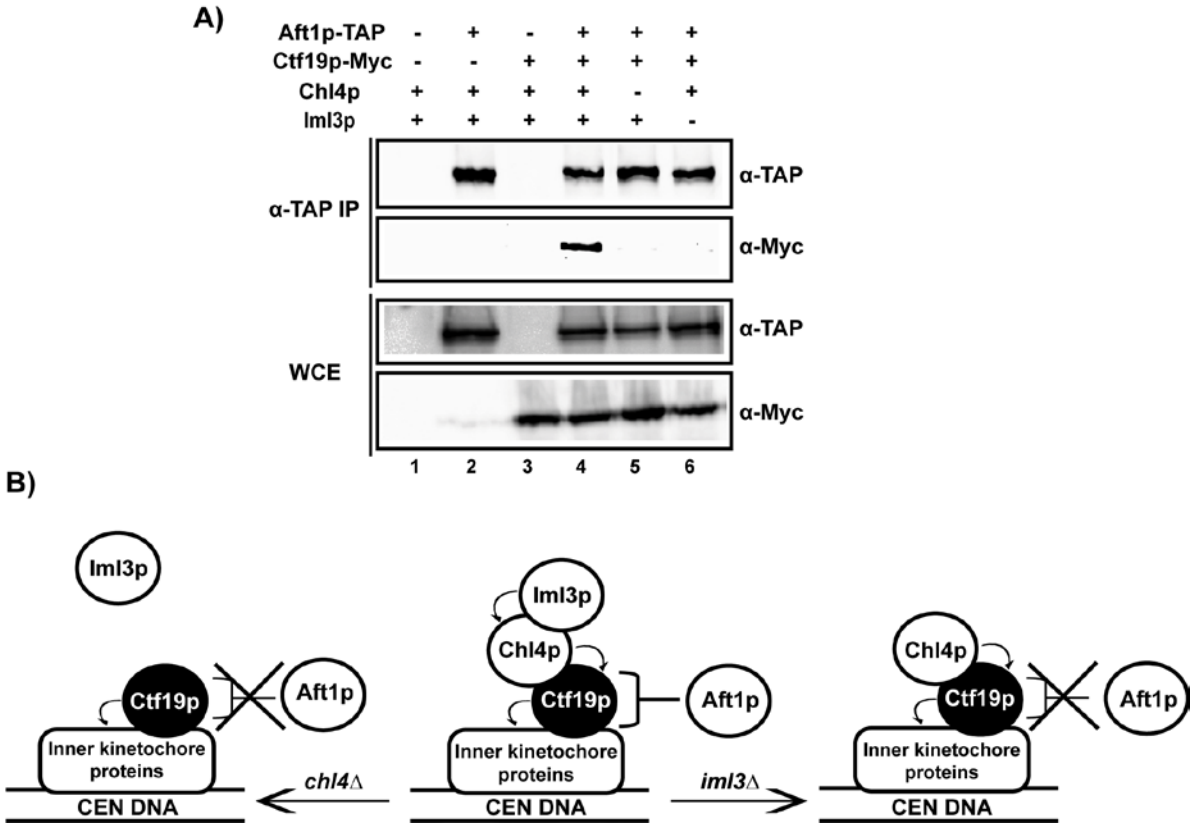

A)
Aft1p-TAP - + - + + +
Ctf19p-Myc - - + + + +
Chl4p + + + + - +
Iml3p + + + + + -
α-TAP IP
α-TAP
α-Myc
WCE
α-TAP
α-Myc
1 2 3 4 5 6
B)
Iml3p
Ctf19p
Aft1p
Inner kinetochore proteins
CEN DNA
chl4Δ
Iml3p
Chl4p
Ctf19p
Aft1p
Inner kinetochore proteins
CEN DNA
iml3Δ
Chl4p
Ctf19p
Aft1p
Inner kinetochore proteins
CEN DNA

Figure 8: Aft1p interaction with the kinetochore protein Chl4p is dependent on Iml3p but not Ctf19p. (A) Anti-TAP immunoprecipitations were performed with strains containing different combinations of TAP-tagged Aft1p and Myc-tagged Chl4p in the presence or absence of Ctf19p and Iml3p. 10% of the eluate was probed for anti-TAP while 90% of the eluate was probed for anti-Myc. **(B)** Schematic representation illustrating the Aft1p-Chl4p interaction in the presence or absence of Ctf19p and Iml3p. (IP: immunoprecipitations; WCE: whole-cell extract)

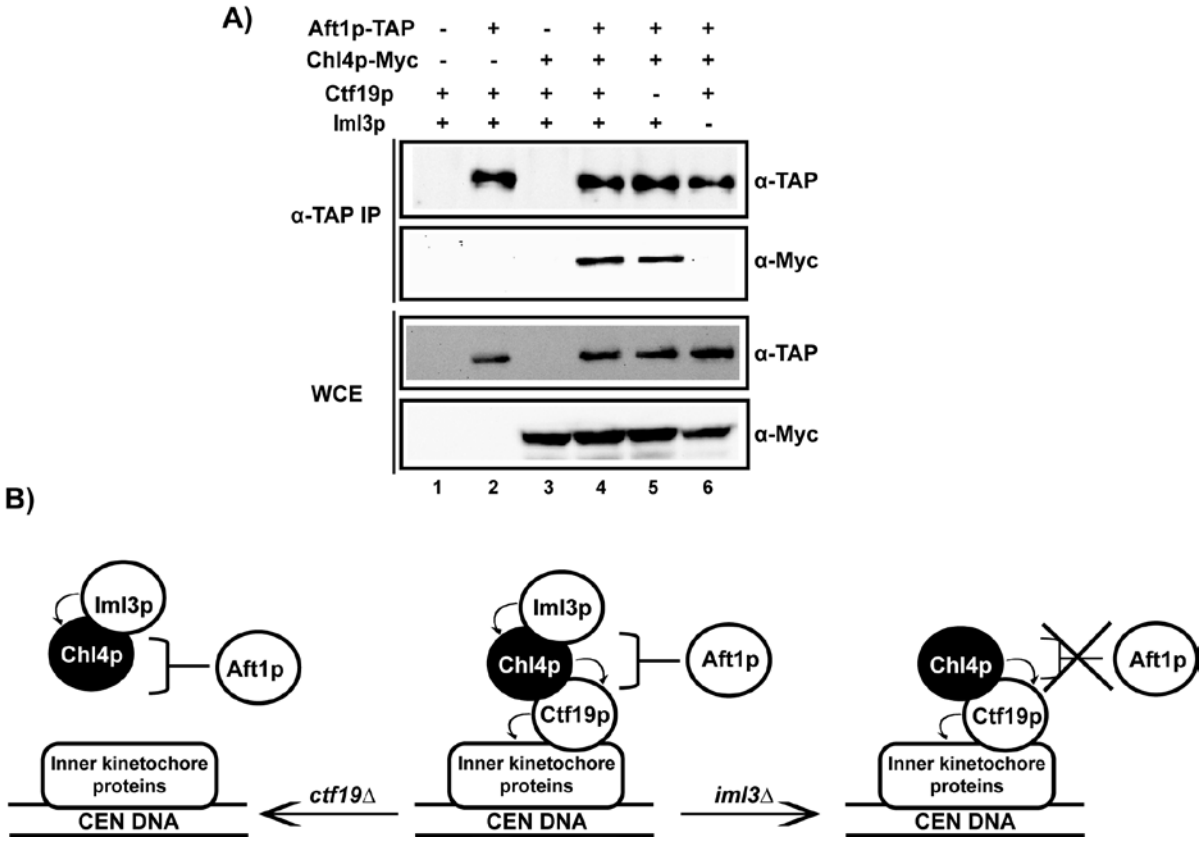
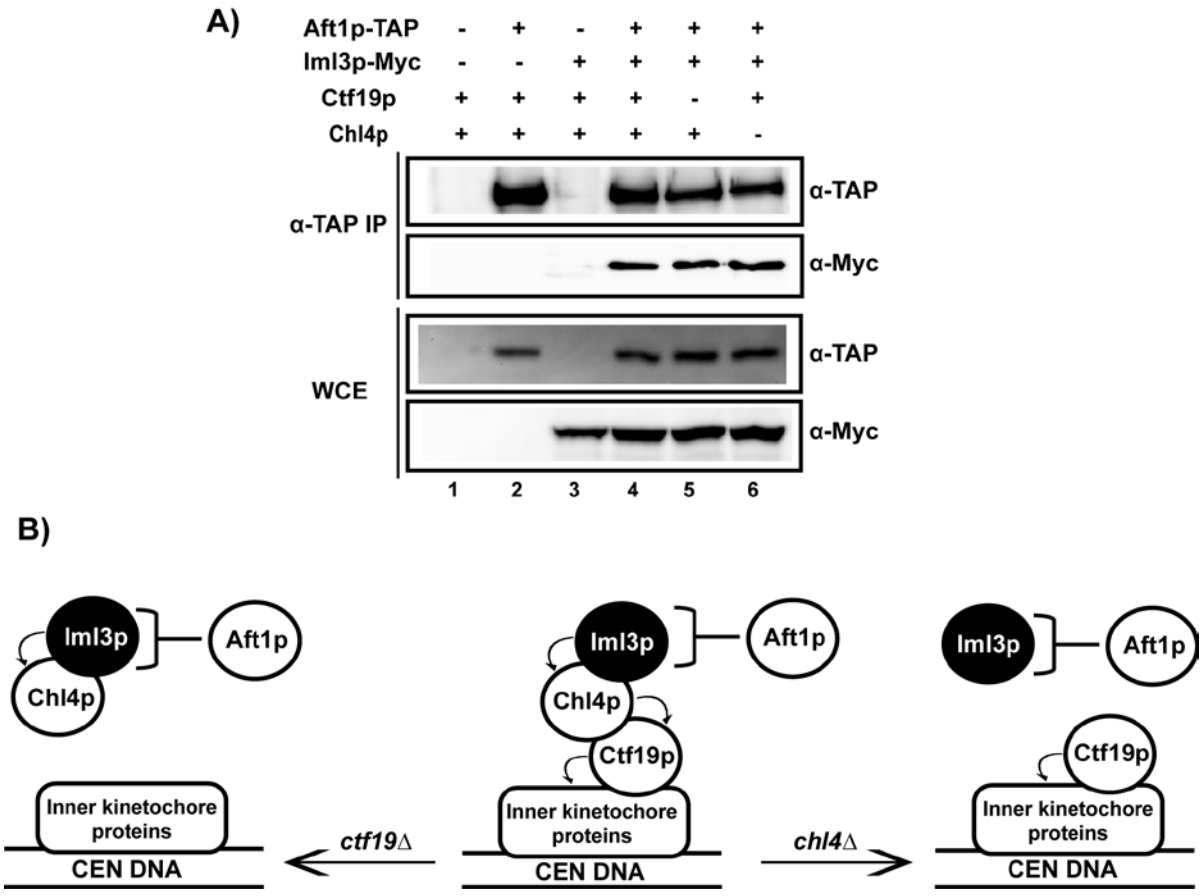

A)
Aft1p-TAP - + - + + +
Chl4p-Myc - - + + + +
Ctf19p + + + + - +
Iml3p + + + + + -
α-TAP IP
α-TAP
α-Myc
WCE
α-TAP
α-Myc
1 2 3 4 5 6
B)
Iml3p
Chl4p
Aft1p
Inner kinetochore proteins
CEN DNA
ctf19Δ
Ctf19p
iml3Δ

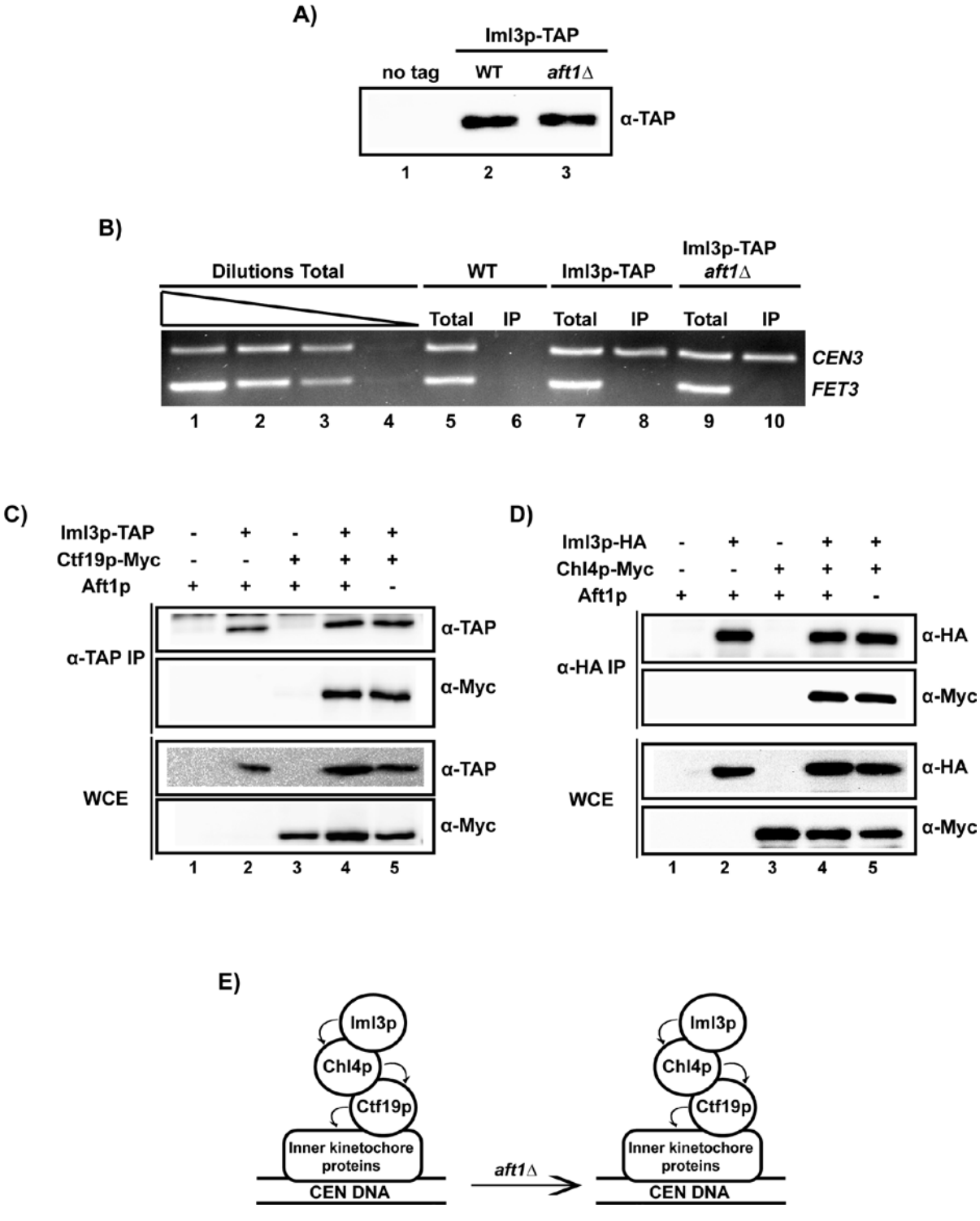
Figure 9: Aft1p interaction with the kinetochore protein Iml3p is independent of Ctf19p and Chl4p. (A) Anti-TAP immunoprecipitations were performed with strains containing different combinations of TAP-tagged Aft1p and Myc-tagged Iml3p in the presence and/or absence of Ctf19p and Chl4p. 10% of the eluate was probed for anti-TAP while 90% of the eluate was probed for anti-Myc. **(B)** Schematic representation illustrating the Aft1p-Iml3p interaction in the presence or absence of Ctf19p and Chl4p. (IP: immunoprecipitations; WCE: whole-cell extract)


A)
Aft1p-TAP - + - + + +
Iml3p-Myc - - + + + +
Ctf19p + + + + - +
Chl4p + + + + + -
α-TAP IP
α-TAP
α-Myc
WCE
α-TAP
α-Myc
1 2 3 4 5 6
B)
Iml3p
Aft1p
Chl4p
Inner kinetochore proteins
CEN DNA
ctf19Δ
Ctf19p
chl4Δ

Aft1p does not impact Iml3p kinetochore localization.

To determine if Aft1p impacts the localization of Iml3p to the centromere, I performed a chromatin-immunoprecipitation (ChIP) assay using an untagged control, Iml3p-TAP, and Iml3p-TAP *aft1Δ* strains (Figure 10A). TAP-tagged Iml3p was immunoprecipitated from formaldehyde cross-linked whole cell extracts and co-precipitated DNA was subjected to multiplex PCR with primers specific to the centromere region of chromosome 3 (*CEN3*) and the negative control (*FET3*). My results indicate that Aft1p does not impact the centromeric localization of Iml3p (Figure 10B). To further confirm my results, I carried out protein co-immunoprecipitation experiments to investigate if Iml3p-Ctf19p and Iml3p-Chl4p interactions are dependent on Aft1p. My results demonstrate that the Iml3p-Ctf19p and Iml3p-Chl4p interactions are not disrupted in an *aft1Δ* background (Figure 10C and D). Taken together, my results indicate that Aft1p is not regulating Iml3p localization to centromeres or interaction with COMA proteins (Figure 10E).

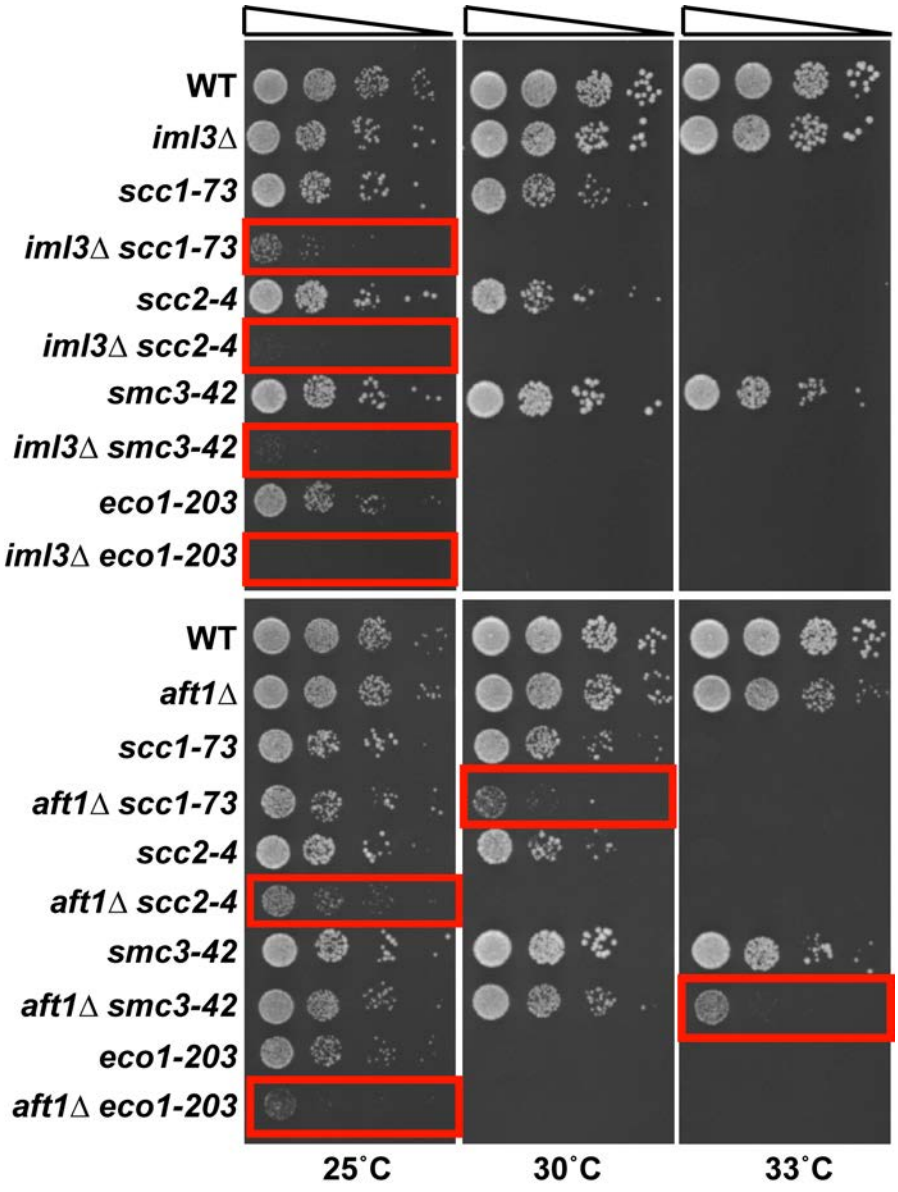
Figure 10: Aft1p has no impact on the kinetochore localization and protein interactions of Iml3p. (A) Anti-TAP immunoprecipitation of Iml3p-TAP in the presence and absence of Aft1p was followed by ChIP analysis. **(B)** Traditional ChIP performed using untagged, Iml3p-TAP, and Iml3p-TAP *aft1*Δ strains demonstrate that Iml3p localization to the centromere is not affected by Aft1p. Total or immunoprecipitated (IP) DNA was subjected to multiplex PCR amplification using primers specific to the centromere of chromosome 3 (*CEN3*) and the negative control *FET3*. **(C)** Iml3p-Ctf19p interaction is not affected by Aft1p. Anti-TAP immunoprecipitations were performed with strains containing different combinations of TAP-tagged Iml3p and Myc-tagged Ctf19p in the presence or absence of Aft1p. **(D)** Iml3p-Chl4p interaction is not affected by Aft1p. Anti-HA immunoprecipitations were performed with strains containing different combinations of HA-tagged Iml3p and Myc-tagged Chl4p in the presence or absence of Aft1p. **(E)** Schematic representation illustrating Iml3p kinetochore localization and protein interactions in the presence or absence of Aft1p. (IP: immunoprecipitations; WCE: whole-cell extract)


A)
Iml3p-TAP
no tag
WT
aft1Δ
α-TAP
1
2
3
B)
Dilutions Total
WT
Iml3p-TAP
Iml3p-TAP
aft1Δ
Total
IP
Total
IP
Total
IP
CEN3
FET3
1
2
3
4
5
6
7
8
9
10
C)
Iml3p-TAP - + - + +
Ctf19p-Myc - - + + +
Aft1p + + + + -
α-TAP IP
α-TAP
α-Myc
WCE
α-TAP
α-Myc
1
2
3
4
5
D)
Iml3p-HA - + - + +
Chl4p-Myc - - + + +
Aft1p + + + + -
α-HA IP
α-HA
α-Myc
WCE
α-HA
α-Myc
1
2
3
4
5
E)
Iml3p
Chl4p
Ctf19p
Inner kinetochore proteins
CEN DNA
aft1Δ
Iml3p
Chl4p
Ctf19p
Inner kinetochore proteins
CEN DNA

aft1Δ and iml3Δ mutants display negative genetic interactions with members of the sister chromatid cohesion pathway.

Given that the only known role for Iml3p has been to establish pericentric cohesion (Fernius and Marston, 2009; Ghosh et al., 2004; Kiburz et al., 2005; Marston et al., 2004), and that genetic interactions predict functional relationships (Tong et al., 2001), I wanted to determine whether *aft1Δ* and *iml3Δ* mutants display synthetic genetic interactions with mutants of the sister chromatid cohesion pathway. I chose four temperature-sensitive mutants of the cohesion pathway that retain partial function at permissive temperatures but are lethal at higher temperatures: *scc1-73*, *scc2-4*, *smc3-42* (Michaelis et al., 1997), and *eco1-203* (Skibbens et al., 1999). Scc1p and Smc3p are part of the tri-partite cohesin ring that physically links sister chromatids until the onset of anaphase. Scc2p is part of the Scc2p/Scc4p cohesin loader complex, whereas Eco1p functions through acetylation of Smc3p to establish sister chromatid cohesion (Skibbens, 2010). As expected, I found that *iml3Δ* mutants display negative genetic interactions with all the temperature-sensitive cohesion mutants at 25°C. Similarly, I found that *aft1Δ* in combination with the cohesion mutants also resulted in synthetic genetic interactions, however the strength of the interactions varied by temperature and were not as pronounced as those with *iml3Δ* (Figure 11). This suggests that like Iml3p, Aft1p may have a role in sister chromatid cohesion.

Figure 11: *aft1Δ* and *iml3Δ* mutants display negative genetic interactions with temperature-sensitive mutants of the cohesion pathway. Cells were plated in ten-fold serial dilutions (OD_{600} = 0.1, 0.01, 0.001, 0.0001) onto YPD plates and incubated for two days at the indicated temperatures. Red boxes highlight the negative genetic interactions between *aft1Δ*, *iml3Δ*, and the cohesion mutants.

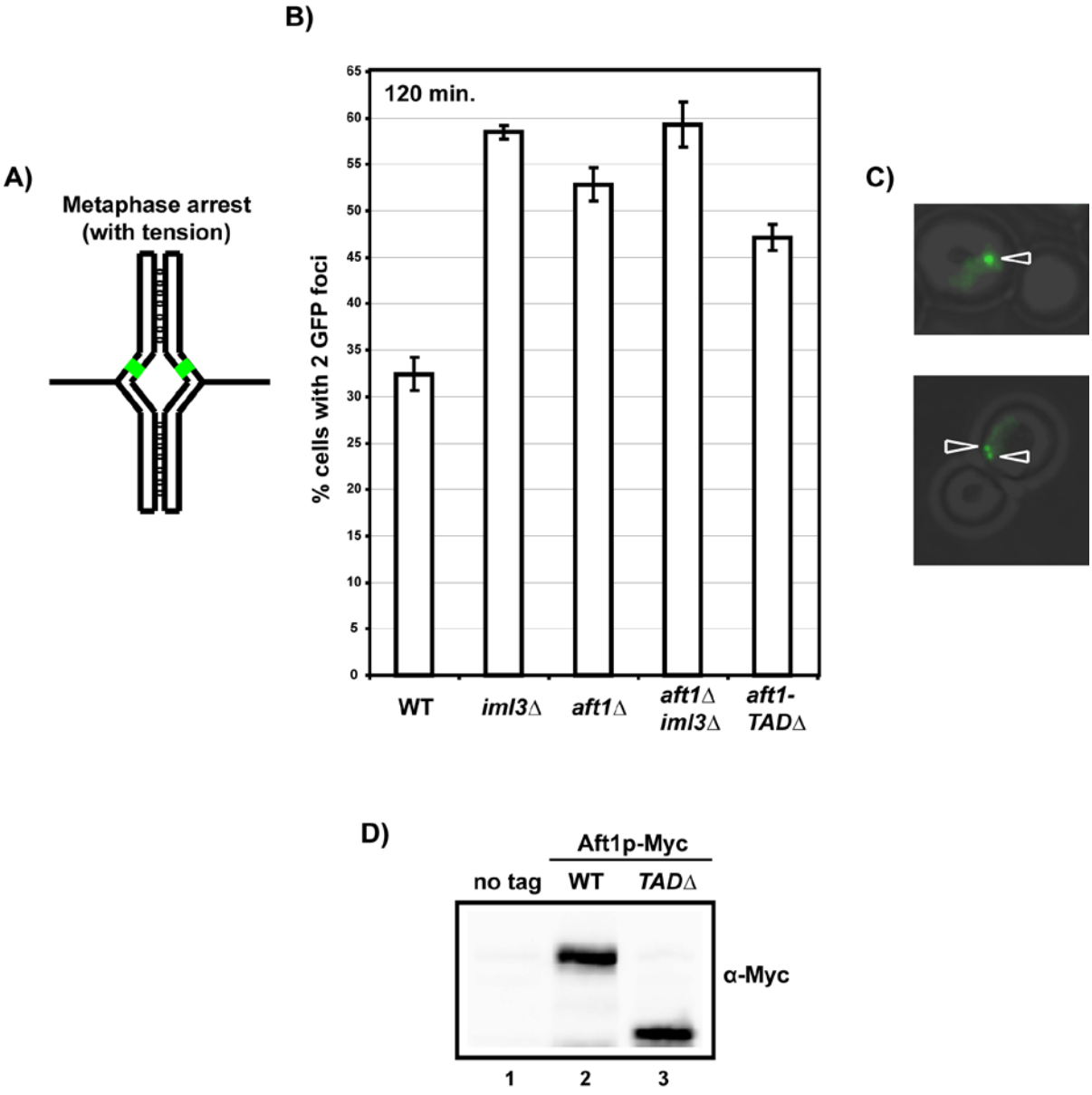

WT
iml3Δ
scc1-73
iml3Δ scc1-73
scc2-4
iml3Δ scc2-4
smc3-42
iml3Δ smc3-42
eco1-203
iml3Δ eco1-203
WT
aft1Δ
scc1-73
aft1Δ scc1-73
scc2-4
aft1Δ scc2-4
smc3-42
aft1Δ smc3-42
eco1-203
aft1Δ eco1-203
25˚C
30˚C
33˚C

aft1Δ mutants exhibit pericentromeric cohesion defects in mitosis and this is dependent on its transactivation domain.

It has been previously shown that Iml3p and other Ctf19 complex proteins exhibit pericentromeric cohesion defects in mitosis (Fernius and Marston, 2009). The pericentromere is a cohesin enriched ~50kb domain surrounding the ~120bp centromere (Glynn et al., 2004; Kiburz et al., 2005; Lengronne et al., 2004; Weber et al., 2004). To determine whether Aft1p displays similar defects in mitotic chromosomes, I used strains that express TetR-GFP and have *tetO* arrays integrated 2.4 kb from the centromere of chromosome 4 (+2.4*CEN4-GFP*) and asked if sister chromatids are more frequently separated in a metaphase arrest (Figure 12A). Wild-type, *iml3Δ*, *aft1Δ*, and *aft1Δiml3Δ* cells under the control of a methionine-repressible promoter (MET-*CDC20*) were released from a G1 alpha-factor block into methionine rich-media. This induces a metaphase arrest through depletion of Cdc20p (required for the metaphase to anaphase transition) (Uhlmann et al., 2000). Sister chromatids in this metaphase arrest remain under tension from the microtubule pulling forces (Figure 12A) and the separation of sister chromatids were scored by counting the number of cells that have 1 or 2 GFP foci (Figure 12C). Consistent with previous studies, my results show that approximately 30% of wild-type cells exhibit GFP dot separation; whereas this proportion increases to 60% in *iml3Δ* cells indicating that *iml3Δ* mutants exhibit pericentromeric cohesion defects (Figure 12B) (Fernius and Marston, 2009). I further demonstrate that approximately 55% of *aft1Δ* cells have separated GFP foci indicating that Aft1p, like Iml3p, is required for establishing pericentromeric cohesion in mitosis. As *aft1Δiml3Δ* cells did not display additive defects, it suggests that Aft1p and Iml3p function together to regulate pericentromeric cohesion (Figure 12B). I was then interested to determine which domain of Aft1p is responsible for this phenotype. Previous studies have

mapped the transactivation domain of Aft1p to the C-terminal region and within amino acids 413-690 (Yamaguchi-Iwai et al., 2002), while the N-terminal region is believed to function as the DNA binding domain (Yamaguchi-Iwai et al., 1995). As such, I deleted the transactivation domain (TAD) of Aft1p, or amino acids 413-690, to create the truncated *aft1-TADΔ* (Figure 12D) and asked whether we observe the same phenotype as *aft1Δ* cells. My results show that the *aft1-TADΔ* strain displays pericentric cohesion defects closely matching *aft1Δ* cells indicating that the transactivation domain of Aft1p may play the dominant role in the pericentric cohesion pathway (Figure 12B).

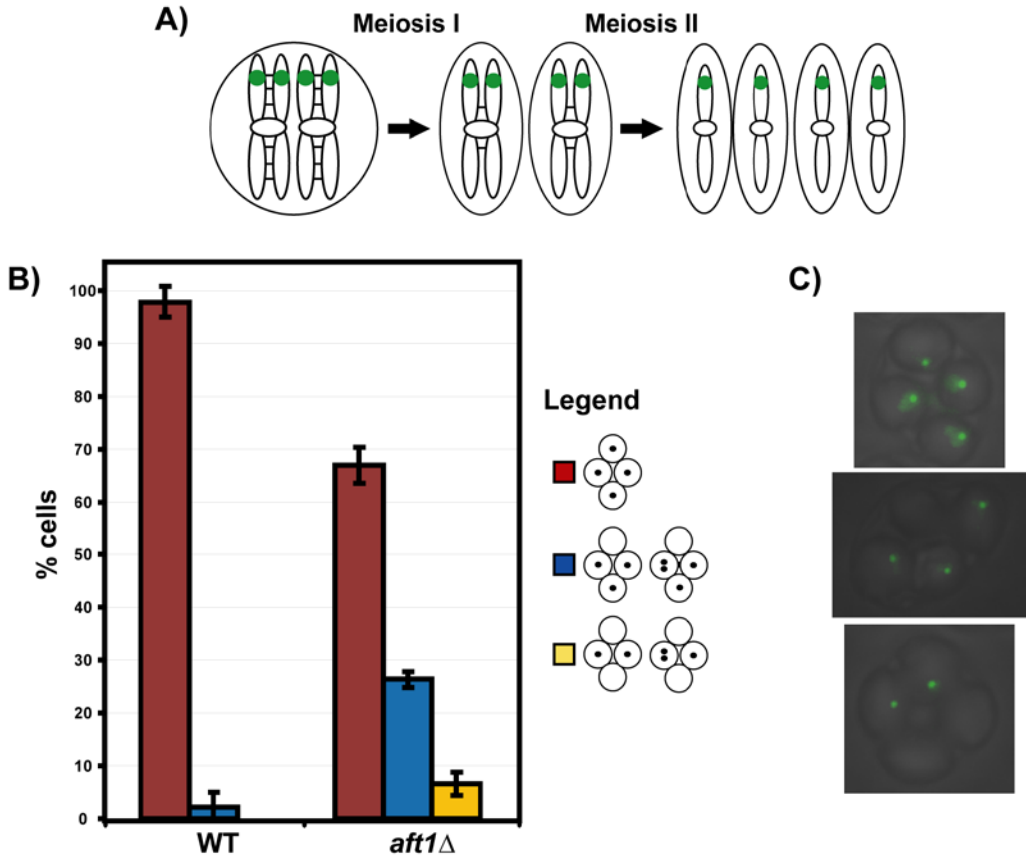
Figure 12: *aft1Δ* and *iml3Δ* mutants exhibit pericentromeric cohesion defects in mitosis. **(A)** Depletion of Cdc20p induces a metaphase arrest while maintaining tension between sister chromatids after which the frequency of GFP separation was assayed by microscopy (+2.4*CEN4-GFP*; 2.4 kb to the right of *CEN4*). **(B)** Chromatids are more frequently separated in *aft1Δ*, *iml3Δ* and *aft1-TADΔ* mutants. Results are the mean of three experiments in which 200 cells were scored 120 minutes after release from G1, in a metaphase arrest. **(C)** A snapshot of cells at the 120 minute mark depicting sister chromatid separation. **(D)** Whole cell extract expression of Myc-tagged Aft1p and truncated Myc-tagged *aft1-TADΔ*.


A)
Metaphase arrest
(with tension)
B)
120 min.
% cells with 2 GFP foci
65
60
55
50
45
40
35
30
25
20
15
10
5
0
WT
iml3Δ
aft1Δ
aft1Δ iml3Δ
aft1-TADΔ
C)
D)
Aft1p-Myc
no tag
WT
TADΔ
α-Myc
1
2
3

aft1Δ mutants exhibit pericentromeric cohesion defects in meiosis.

After the first meiotic division in which homologous chromosomes are segregated, sister chromatids lack all arm cohesion and depend only on pericentromeric cohesion (Marston and Amon, 2004). As such, defects in pericentromeric cohesion results in chromosome non-disjunction following the second meiotic division. Previous reports have shown that *iml3Δ* mutants display pericentromeric cohesion defects in meiosis (Fernius and Marston, 2009; Ghosh et al., 2004; Kiburz et al., 2005; Marston et al., 2004). Genome-wide screens have also identified *aft1Δ* as having mild chromosome segregation defects during meiosis (Marston et al., 2004), suggesting Aft1p may also play a role in meiotic pericentromeric cohesion. To further examine this, I monitored the fate of GFP labelled chromosomes in wild-type and *aft1Δ* diploids after meiosis (Figure 13A). The strains used have GFP labels located at *URA3* (38.4 kb from *CEN5*) on both copies of chromosome 5 (tetR-GFP::LEU2 URA3::tetOx224). Using the same strains, a previous report showed the following GFP segregation pattern for *iml3Δ* mutants: approximately 60% had GFP dots in all 4 spores, ~32% in 3 out of 4 spores, and ~8% in 2 out of 4 spores (Fernius and Marston, 2009). My results indicate that *aft1Δ* mutants display GFP-dot segregation patterns similar to *iml3Δ* mutants after meiosis (Figure 13B and C). I demonstrate that *aft1Δ* mutants have the following GFP segregation pattern: ~65% had GFP dots in all 4 spores, ~27% in 3 out of 4 spores, and ~8% in 2 out of 4 spores. In comparison, ~100% of wild-type diploids had GFP dots in all 4 spores following sporulation indicating proper chromosome segregation. Therefore, Aft1p, like Iml3p, is required for proper chromosome segregation during meiosis.

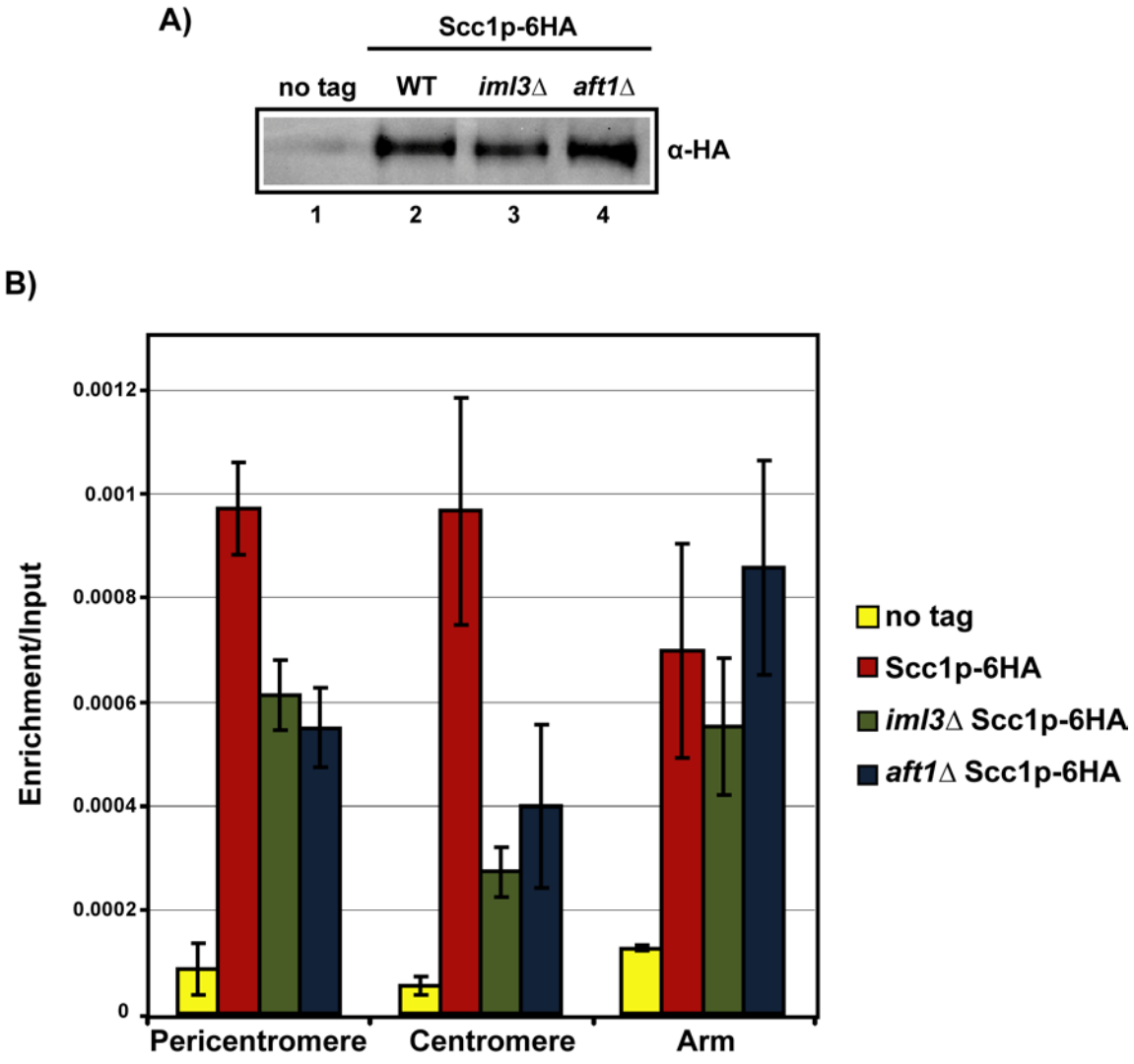
Figure 13: Aft1p is required for proper chromosome segregation during meiosis. (A) Wildtype (WT) and *aft1*Δ diploids that have GFP-labelled chromosome 5 (*tetR-GFP::LEU2 URA3::tetOx224*) at *URA3* (38.4 kb from the centromere) were induced to sporulate at 30°C. **(B)** GFP dot segregation patterns in tetra-nucleate cells were scored and represented as percentages. Results are the mean of three experiments in which 100 tetra-nucleate cells were scored for GFP segregation patterns. **(C)** A snapshot of cells exhibiting different GFP dot segregation patterns.


A)
Meiosis I
Meiosis II
B)
% cells
100
90
80
70
60
50
40
30
20
10
0
WT
aft1Δ
Legend
C)

Aft1p and Iml3p are required for increased cohesin at the centromere and pericentromere.

Increased separation of sister chromatids at the pericentromere are indicative of cohesion defects that are required to resist the microtubule pulling forces (Fernius and Marston, 2009). To assess whether Aft1p is required for increased cohesin binding at the pericentromere, I employed chromatin immunoprecipitation (ChIP) followed by real-time quantitative PCR (qPCR) to examine the enrichment level of the cohesin subunit Scc1p-HA at three different sites on chromosome 4. The three sites chosen were previously shown to be enriched for cohesin, and are located at the chromosomal arm, pericentromere, and centromere regions (Fernius and Marston, 2009). Wild-type, *iml3Δ*, and *aft1Δ* cells that express HA-tagged Scc1p (and an untagged control) (Figure 14A) were arrested in metaphase following treatment with the microtubule depolymerizing drugs nocodazole and benomyl. This generated a metaphase arrest whereby sister chromatids are not under tension from the microtubule pulling forces. HA-tagged Scc1p was then immunoprecipitated from formaldehyde cross-linked extracts upon which the co-precipitated DNA was subjected to qPCR analysis and enrichment/input values were calculated as described (Fernius and Marston, 2009). My results indicate that there is reduced Scc1p enrichment at the pericentromere and centromere regions in *iml3Δ* and *aft1Δ* mutants as compared to wild-type cells (Figure 14B). In contrast, Scc1p levels were comparable between wild-type, *iml3Δ*, and *aft1Δ* cells at the chromosomal arm location. Therefore, Aft1p and Iml3p share the same role in promoting cohesin association at the centromere and pericentromere.

Figure 14: Levels of Scc1p-6HA are reduced in *aft1Δ* and *iml3Δ* mutants at the pericentromere and centromere but not at the chromosomal arm. **(A)** Anti-HA immunoprecipitation of Scc1p-6HA in wild-type, *aft1Δ,* and *iml3Δ* mutants was followed by qPCR. **(B)** qPCR analysis of Scc1p-6HA levels was performed at three different regions of chromosome 4. Cells were arrested in metaphase of mitosis in the absence of microtubules following treatment with the microtubule depolymerizing drugs nocodazole and benomyl. Results are the mean of three experiments.


A)
Scc1p-6HA
no tag
WT
iml3Δ
aft1Δ
α-HA
1
2
3
4
B)
Enrichment/Input
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
Pericentromere
Centromere
Arm
no tag
Scc1p-6HA
iml3Δ Scc1p-6HA
aft1Δ Scc1p-6HA

Chapter 4: DISCUSSION

Several genome-wide studies identified Aft1p as a potential regulator in chromosome stability (Berthelet et al., 2010; Marston et al., 2004; Measday et al., 2005; Wong et al., 2007; Yu et al., 2008; Yuen et al., 2007). In this study, I confirm that Aft1p interacts with kinetochore proteins Ctf19p and Chl4p through Iml3p. Furthermore, through a combination of genetics, chromatin immunoprecipitation (ChIP) experiments and fluorescence imaging data, I demonstrate that Aft1p, like members of the Ctf19 complex, functions in mitotic and meiotic sister chromatid cohesion at the pericentromere and centromere through the increased loading of cohesin.

Iml3p, Aft1p, and sister chromatid cohesion

The specific function of Ctf19p, Chl4p, and Iml3p within the Ctf19 kinetochore complex remains unknown. Nor is it understood how each member of the Ctf19 complex contributes to cohesin loading at the centromere and pericentromere. It is suggested that one of the functions of Ctf19p and Mcm21p in the cohesion pathway is the recruitment of the more peripheral members Chl4p and Iml3p which play the primary role in cohesin loading (Fernius and Marston, 2009). This argument is based on results demonstrating that single and double Ctf19 complex mutants display similar cohesion defects (Fernius and Marston, 2009), and that Iml3p is the most peripheral Ctf19 complex subunit (Pot et al., 2003). While Iml3p might be the key subunit directing cohesin loading at the centromere, a physical interaction between Iml3p and cohesin or the Scc2p/Scc4p loader complex has not been reported. However, the fact that Aft1p interaction with the kinetochore is through Iml3p (Figure 7, 8, and 9), and that mitotic sister chromatid cohesion defects displayed by *aft1Δiml3Δ* mutant cells are comparable to the single mutants (Figure 12B) suggests that one role Iml3p may be playing is recruiting additional factors, such as Aft1p, to the centromere. However, the role

of Iml3p in the cohesion pathway may not be limited to just recruitment of Aft1p. We can infer from the growth assays that Iml3p plays additional roles in the cohesion pathway not shared by Aft1p as the strength of the genetic interactions of *aft1Δ* mutants with the cohesion mutants were not as pronounced as *iml3Δ* mutants (Figure 11).

Does Aft1p have a transcriptional or regulatory role in chromosome stability?

How could Aft1p be influencing sister chromatid cohesion? Although Aft1p has not been linked to the transcription of genes implicated in chromosome stability or benomyl resistance (Berthelet et al., 2010), we cannot eliminate the possibility that some of Aft1p's role in chromosome stability is mediated through transcription. That said, the physical interaction between Aft1p and Iml3p and the co-localization of Aft1p to kinetochore proteins strongly argues for a role for Aft1p directly at the kinetochore. Aft1p has not been identified in the numerous proteomic studies of the kinetochore (discussed in (McAinsh et al., 2003; Westermann et al., 2007)) but only via two-hybrid screens (Wong et al., 2007; Yu et al., 2008) suggesting that Aft1p interaction with the kinetochore may be transient (Bruckner et al., 2009). Indeed, I may have been able to detect the Aft1p-Iml3p interaction because my purifications utilized low-speed centrifugation which has been shown to increase the solubility of chromatin associated proteins (Lambert et al., 2010).

What are the possible roles for Aft1p at the kinetochore? I have shown that Aft1p is not impacting Iml3p localization to the centromere nor Ctf19 complex integrity (Figure 10). One possibility is that Aft1p is physically interacting with and recruiting members of the cohesin complex or the Scc2p/Scc4p loader complex to the centromere; however, no physical interaction between Aft1p and these subunits has been reported. An alternative possibility is that the kinetochore is harnessing the ability of transcription factors to recruit chromatin remodelers or modifiers which are necessary to specifically position or add

epigenetic marks to pericentromeric histones which in turn recruit Scc2p/Scc4p. There is precedent for this idea as during DNA damage repair, cohesin is enriched at sites flanking the DNA break in a manner dependent on histone H2A phosphorylation (Strom et al., 2004; Unal et al., 2004). Moreover, histone modifications dependent on a functional kinetochore have been reported at the centromere and these modifications contribute to chromosome segregation (Choy et al., 2011). Furthermore, establishment of cohesion has been linked to numerous chromatin regulators and histone modifications (Baetz et al., 2004; Hakimi et al., 2002; Huang et al., 2004; Ogiwara et al., 2007; Oum et al., 2011) and chromatin-remodeling complexes were also found to interact with kinetochore proteins and localize to the centromere (Gkikopoulos et al., 2011; Hsu et al., 2003; Wong et al., 2007). Interestingly, yeast-two hybrid and co-immunoprecipitation experiments have shown a physical interaction between NIPBL (human ortholog of Scc2p) and histone deacetylases (HDAC 1 and HDAC 3) (Jahnke et al., 2008). Nevertheless, the exact interplay between pericentromeric chromatin structure, cohesion, and histone modifications remains unknown. Additionally, it is not known whether chromatin remodelers/modifiers are enriched at cohesin loading sites at a global level (Riedel et al., 2004). Affinity-capture mass spectrometry studies have demonstrated Aft1p co-purifying with protein subunits of the SWI/SNF chromatin remodeler (Lambert et al., 2010), and the lysine acetyltransferases SAGA (Lee et al., 2011) and NuA4 (Lambert et al., 2010); all three which have been implicated in chromosome stability and cohesion defects (Gkikopoulos et al., 2011; Krogan et al., 2004; Marston et al., 2004). In fact, the catalytic subunits and some members of the SWI/SNF, SAGA and NuA4 complexes were shown to be required for proper chromosome segregation during meiosis in a manner resembling *aft1Δ* and *iml3Δ* mutants (Marston et al., 2004). Therefore, a possible model explaining the Aft1p-kinetochore interaction is that the Ctf19 complex through its Iml3p

subunit recruits Aft1p which in turn recruits chromatin remodelers and modifiers that impact cohesin loading across the centromere and pericentromere. The fact that the transactivation domain of Aft1p, whom one would speculate is recruiting chromatin remodelers and modifiers to iron-regulon promoters (Yamaguchi-Iwai et al., 2002), has been shown to play the dominant role in pericentromeric cohesion (Figure 12B) adds support to this proposed function for Aft1p at the kinetochore. As numerous other transcription factors have been shown to interact with kinetochore proteins (Wong et al., 2007), the role of transcription factors in pericentromeric cohesion may not be limited to Aft1p, but rather to a general mechanism that recruits remodelers to specific loci. On the other hand, the physical interaction between Aft1p and chromatin remodelers could possibly be independent of Aft1p's role at the kinetochore and solely related to its transcriptional role in iron regulation. Additional studies are required to further elucidate this proposed mechanism for Aft1p at the kinetochore. For example, co-immunoprecipitation experiments could prove a physical interaction between Iml3p and chromatin remodelers that is dependent on Aft1p or its transactivation domain.

Conclusion

The objective of this study was to decipher the role of the iron-responsive transcription factor, Aft1p, in chromosome stability. I have demonstrated that Aft1p physically interacts with the Ctf19 kinetochore complex through its most peripheral subunit, Iml3p. I have further shown that Aft1p functions in the cohesion pathway in mitosis and meiosis and displays cohesion defects that mimic *iml3Δ* mutants. Specifically, I have shown that Aft1p is required for the increased loading of the cohesin complex along the centromere and pericentromere. Further characterization of the Aft1p-kinetochore-cohesion interplay can help in our understanding of this elusive pathway.

REFERNCES

Baetz, K.K., Krogan, N.J., Emili, A., Greenblatt, J., and Hieter, P. (2004). The ctf13-30/CTF13 genomic haploinsufficiency modifier screen identifies the yeast chromatin remodeling complex RSC, which is required for the establishment of sister chromatid cohesion. Mol Cell Biol *24*, 1232-1244.

Bardhan, A. (2010). Many functions of the meiotic cohesin. Chromosome Res *18*, 909-924.

Beckouet, F., Hu, B., Roig, M.B., Sutani, T., Komata, M., Uluocak, P., Katis, V.L., Shirahige, K., and Nasmyth, K. (2010). An Smc3 acetylation cycle is essential for establishment of sister chromatid cohesion. Mol Cell *39*, 689-699.

Berthelet, S., Usher, J., Shulist, K., Hamza, A., Maltez, N., Johnston, A., Fong, Y., Harris, L.J., and Baetz, K. (2010). Functional genomics analysis of the Saccharomyces cerevisiae iron responsive transcription factor Aft1 reveals iron-independent functions. Genetics *185*, 1111-1128.

Blaiseau, P.L., Lesuisse, E., and Camadro, J.M. (2001). Aft2p, a novel iron-regulated transcription activator that modulates, with Aft1p, intracellular iron use and resistance to oxidative stress in yeast. J Biol Chem *276*, 34221-34226.

Bloom, K., Sharma, S., and Dokholyan, N.V. (2006). The path of DNA in the kinetochore. Curr Biol *16*, R276-278.

Bruckner, A., Polge, C., Lentze, N., Auerbach, D., and Schlattner, U. (2009). Yeast two-hybrid, a powerful tool for systems biology. Int J Mol Sci *10*, 2763-2788.

Casas, C., Aldea, M., Espinet, C., Gallego, C., Gil, R., and Herrero, E. (1997). The AFT1 transcriptional factor is differentially required for expression of high-affinity iron uptake genes in Saccharomyces cerevisiae. Yeast *13*, 621-637.

Chen, O.S., Crisp, R.J., Valachovic, M., Bard, M., Winge, D.R., and Kaplan, J. (2004). Transcription of the yeast iron regulon does not respond directly to iron but rather to iron-sulfur cluster biosynthesis. J Biol Chem *279*, 29513-29518.

Choy, J.S., Acuna, R., Au, W.C., and Basrai, M.A. (2011). A role for histone H4K16 hypoacetylation in Saccharomyces cerevisiae kinetochore function. Genetics *189*, 11-21.

Courel, M., Lallet, S., Camadro, J.M., and Blaiseau, P.L. (2005). Direct activation of genes involved in intracellular iron use by the yeast iron-responsive transcription factor Aft2 without its paralog Aft1. Mol Cell Biol *25*, 6760-6771.

De Wulf, P., McAinsh, A.D., and Sorger, P.K. (2003). Hierarchical assembly of the budding yeast kinetochore from multiple subcomplexes. Genes Dev *17*, 2902-2921.

Dubacq, C., Chevalier, A., Courbeyrette, R., Petat, C., Gidrol, X., and Mann, C. (2006). Role of the iron mobilization and oxidative stress regulons in the genomic response of yeast to hydroxyurea. Mol Genet Genomics *275*, 114-124.

Eckert, C.A., Gravdahl, D.J., and Megee, P.C. (2007). The enhancement of pericentromeric cohesin association by conserved kinetochore components promotes high-fidelity chromosome segregation and is sensitive to microtubule-based tension. Genes Dev *21*, 278-291.

Fernius, J., and Marston, A.L. (2009). Establishment of cohesion at the pericentromere by the Ctf19 kinetochore subcomplex and the replication fork-associated factor, Csm3. PLoS Genet *5*, e1000629.

Foury, F., and Talibi, D. (2001). Mitochondrial control of iron homeostasis. A genome wide analysis of gene expression in a yeast frataxin-deficient strain. J Biol Chem *276*, 7762-7768.

Gerkes, E.H., van der Kevie-Kersemaekers, A.M., Yakin, M., Smeets, D.F., and van Ravenswaaij-Arts, C.M. (2010). The importance of chromosome studies in Roberts syndrome/SC phocomelia and other cohesinopathies. Eur J Med Genet *53*, 40-44.

Ghosh, S.K., Sau, S., Lahiri, S., Lohia, A., and Sinha, P. (2004). The Iml3 protein of the budding yeast is required for the prevention of precocious sister chromatid separation in meiosis I and for sister chromatid disjunction in meiosis II. Curr Genet *46*, 82-91.

Gkikopoulos, T., Singh, V., Tsui, K., Awad, S., Renshaw, M.J., Scholfield, P., Barton, G.J., Nislow, C., Tanaka, T.U., and Owen-Hughes, T. (2011). The SWI/SNF complex acts to constrain distribution of the centromeric histone variant Cse4. EMBO J *30*, 1919-1927.

Glynn, E.F., Megee, P.C., Yu, H.G., Mistrot, C., Unal, E., Koshland, D.E., DeRisi, J.L., and Gerton, J.L. (2004). Genome-wide mapping of the cohesin complex in the yeast Saccharomyces cerevisiae. PLoS Biol *2*, E259.

Guacci, V., Koshland, D., and Strunnikov, A. (1997). A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. Cell *91*, 47-57.

Hakimi, M.A., Bochar, D.A., Schmiesing, J.A., Dong, Y., Barak, O.G., Speicher, D.W., Yokomori, K., and Shiekhattar, R. (2002). A chromatin remodelling complex that loads cohesin onto human chromosomes. Nature *418*, 994-998.

Harbison, C.T., Gordon, D.B., Lee, T.I., Rinaldi, N.J., Macisaac, K.D., Danford, T.W., Hannett, N.M., Tagne, J.B., Reynolds, D.B., Yoo, J., *et al.* (2004). Transcriptional regulatory code of a eukaryotic genome. Nature *431*, 99-104.

Hsu, J.M., Huang, J., Meluh, P.B., and Laurent, B.C. (2003). The yeast RSC chromatin-remodeling complex is required for kinetochore function in chromosome segregation. Mol Cell Biol *23*, 3202-3215.

Hu, B., Itoh, T., Mishra, A., Katoh, Y., Chan, K.L., Upcher, W., Godlee, C., Roig, M.B., Shirahige, K., and Nasmyth, K. (2011). ATP hydrolysis is required for relocating cohesin from sites occupied by its Scc2/4 loading complex. Curr Biol *21*, 12-24.

Huang, J., Hsu, J.M., and Laurent, B.C. (2004). The RSC nucleosome-remodeling complex is required for Cohesin's association with chromosome arms. Mol Cell *13*, 739-750.

Hyland, K.M., Kingsbury, J., Koshland, D., and Hieter, P. (1999). Ctf19p: A novel kinetochore protein in Saccharomyces cerevisiae and a potential link between the kinetochore and mitotic spindle. J Cell Biol *145*, 15-28.

Jahnke, P., Xu, W., Wulling, M., Albrecht, M., Gabriel, H., Gillessen-Kaesbach, G., and Kaiser, F.J. (2008). The Cohesin loading factor NIPBL recruits histone deacetylases to mediate local chromatin modifications. Nucleic Acids Res *36*, 6450-6458.

Jorgensen, P., Nishikawa, J.L., Breitkreutz, B.J., and Tyers, M. (2002). Systematic identification of pathways that couple cell growth and division in yeast. Science *297*, 395-400.

Kaplan, J., McVey Ward, D., Crisp, R.J., and Philpott, C.C. (2006). Iron-dependent metabolic remodeling in S. cerevisiae. Biochim Biophys Acta *1763*, 646-651.

Kiburz, B.M., Reynolds, D.B., Megee, P.C., Marston, A.L., Lee, B.H., Lee, T.I., Levine, S.S., Young, R.A., and Amon, A. (2005). The core centromere and Sgo1 establish a 50-kb cohesin-protected domain around centromeres during meiosis I. Genes Dev *19*, 3017-3030.

Kimura, A., Ohashi, K., and Naganuma, A. (2007). Cisplatin upregulates Saccharomyces cerevisiae genes involved in iron homeostasis through activation of the iron insufficiency-responsive transcription factor Aft1. J Cell Physiol *210*, 378-384.

Klein, F., Mahr, P., Galova, M., Buonomo, S.B., Michaelis, C., Nairz, K., and Nasmyth, K. (1999). A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell *98*, 91-103.

Krogan, N.J., Baetz, K., Keogh, M.C., Datta, N., Sawa, C., Kwok, T.C., Thompson, N.J., Davey, M.G., Pootoolal, J., Hughes, T.R., *et al.* (2004). Regulation of chromosome stability by the histone H2A variant Htz1, the Swr1 chromatin remodeling complex, and the histone acetyltransferase NuA4. Proc Natl Acad Sci U S A *101*, 13513-13518.

Lambert, J.P., Fillingham, J., Siahbazi, M., Greenblatt, J., Baetz, K., and Figeys, D. (2010). Defining the budding yeast chromatin-associated interactome. Mol Syst Biol *6*, 448.

Lange, H., Kaut, A., Kispal, G., and Lill, R. (2000). A mitochondrial ferredoxin is essential for biogenesis of cellular iron-sulfur proteins. Proc Natl Acad Sci U S A *97*, 1050-1055.

Lee, K.K., Sardiu, M.E., Swanson, S.K., Gilmore, J.M., Torok, M., Grant, P.A., Florens, L., Workman, J.L., and Washburn, M.P. (2011). Combinatorial depletion analysis to assemble

the network architecture of the SAGA and ADA chromatin remodeling complexes. Mol Syst Biol *7*, 503.

Lee, T.I., Rinaldi, N.J., Robert, F., Odom, D.T., Bar-Joseph, Z., Gerber, G.K., Hannett, N.M., Harbison, C.T., Thompson, C.M., Simon, I., *et al.* (2002). Transcriptional regulatory networks in Saccharomyces cerevisiae. Science *298*, 799-804.

Lee, W., St Onge, R.P., Proctor, M., Flaherty, P., Jordan, M.I., Arkin, A.P., Davis, R.W., Nislow, C., and Giaever, G. (2005). Genome-wide requirements for resistance to functionally distinct DNA-damaging agents. PLoS Genet *1*, e24.

Lengronne, A., Katou, Y., Mori, S., Yokobayashi, S., Kelly, G.P., Itoh, T., Watanabe, Y., Shirahige, K., and Uhlmann, F. (2004). Cohesin relocation from sites of chromosomal loading to places of convergent transcription. Nature *430*, 573-578.

Li, H., Mapolelo, D.T., Dingra, N.N., Keller, G., Riggs-Gelasco, P.J., Winge, D.R., Johnson, M.K., and Outten, C.E. (2011). Histidine 103 in Fra2 is an iron-sulfur cluster ligand in the [2Fe-2S] Fra2-Grx3 complex and is required for in vivo iron signaling in yeast. J Biol Chem *286*, 867-876.

Lill, R., and Muhlenhoff, U. (2008). Maturation of iron-sulfur proteins in eukaryotes: mechanisms, connected processes, and diseases. Annu Rev Biochem *77*, 669-700.

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

Marston, A.L., and Amon, A. (2004). Meiosis: cell-cycle controls shuffle and deal. Nat Rev Mol Cell Biol *5*, 983-997.

Marston, A.L., Lee, B.H., and Amon, A. (2003). The Cdc14 phosphatase and the FEAR network control meiotic spindle disassembly and chromosome segregation. Dev Cell *4*, 711-726.

Marston, A.L., Tham, W.H., Shah, H., and Amon, A. (2004). A genome-wide screen identifies genes required for centromeric cohesion. Science *303*, 1367-1370.

McAinsh, A.D., Tytell, J.D., and Sorger, P.K. (2003). Structure, function, and regulation of budding yeast kinetochores. Annu Rev Cell Dev Biol *19*, 519-539.

Measday, V., Baetz, K., Guzzo, J., Yuen, K., Kwok, T., Sheikh, B., Ding, H., Ueta, R., Hoac, T., Cheng, B., *et al.* (2005). Systematic yeast synthetic lethal and synthetic dosage lethal screens identify genes required for chromosome segregation. Proc Natl Acad Sci U S A *102*, 13956-13961.

Michaelis, C., Ciosk, R., and Nasmyth, K. (1997). Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell *91*, 35-45.

Mitchell, L., Lambert, J.P., Gerdes, M., Al-Madhoun, A.S., Skerjanc, I.S., Figeys, D., and Baetz, K. (2008). Functional dissection of the NuA4 histone acetyltransferase reveals its role as a genetic hub and that Eaf1 is essential for complex integrity. Mol Cell Biol *28*, 2244-2256.

Musio, A., and Krantz, I.D. (2010). Cohesin biology and the cohesinopathies: Abstracts from the Second Biennial Conference, Pontignano, Italy, 2009. Am J Med Genet A *152A*, 1630-1640.

Nasmyth, K., and Haering, C.H. (2009). Cohesin: its roles and mechanisms. Annu Rev Genet *43*, 525-558.

Ng, T.M., Waples, W.G., Lavoie, B.D., and Biggins, S. (2009). Pericentromeric sister chromatid cohesion promotes kinetochore biorientation. Mol Biol Cell *20*, 3818-3827.

Ocampo-Hafalla, M.T., and Uhlmann, F. (2011). Cohesin loading and sliding. J Cell Sci *124*, 685-691.

Ogiwara, H., Enomoto, T., and Seki, M. (2007). The INO80 chromatin remodeling complex functions in sister chromatid cohesion. Cell Cycle *6*, 1090-1095.

Oliveira, R.A., and Nasmyth, K. (2010). Getting through anaphase: splitting the sisters and beyond. Biochem Soc Trans *38*, 1639-1644.

Oum, J.H., Seong, C., Kwon, Y., Ji, J.H., Sid, A., Ramakrishnan, S., Ira, G., Malkova, A., Sung, P., Lee, S.E., *et al.* (2011). RSC Facilitates Rad59-Dependent Homologous Recombination between Sister Chromatids by Promoting Cohesin Loading at DNA Double-Strand Breaks. Mol Cell Biol *31*, 3924-3937.

Pagani, M.A., Casamayor, A., Serrano, R., Atrian, S., and Arino, J. (2007). Disruption of iron homeostasis in Saccharomyces cerevisiae by high zinc levels: a genome-wide study. Mol Microbiol *65*, 521-537.

Peters, J.M., Tedeschi, A., and Schmitz, J. (2008). The cohesin complex and its roles in chromosome biology. Genes Dev *22*, 3089-3114.

Philpott, C.C., and Protchenko, O. (2008). Response to iron deprivation in Saccharomyces cerevisiae. Eukaryot Cell *7*, 20-27.

Philpott, C.C., Rashford, J., Yamaguchi-Iwai, Y., Rouault, T.A., Dancis, A., and Klausner, R.D. (1998). Cell-cycle arrest and inhibition of G1 cyclin translation by iron in AFT1-1(up) yeast. EMBO J *17*, 5026-5036.

Pot, I., Measday, V., Snydsman, B., Cagney, G., Fields, S., Davis, T.N., Muller, E.G., and Hieter, P. (2003). Chl4p and iml3p are two new members of the budding yeast outer kinetochore. Mol Biol Cell *14*, 460-476.

Riedel, C.G., Gregan, J., Gruber, S., and Nasmyth, K. (2004). Is chromatin remodeling required to build sister-chromatid cohesion? Trends Biochem Sci *29*, 389-392.

Roy, B., and Sanyal, K. (2011). Diversity in Requirement of Genetic and Epigenetic Factors for Centromere Function in Fungi. Eukaryot Cell.

Rutherford, J.C., and Bird, A.J. (2004). Metal-responsive transcription factors that regulate iron, zinc, and copper homeostasis in eukaryotic cells. Eukaryot Cell *3*, 1-13.

Rutherford, J.C., Jaron, S., Ray, E., Brown, P.O., and Winge, D.R. (2001). A second iron-regulatory system in yeast independent of Aft1p. Proc Natl Acad Sci U S A *98*, 14322-14327.

Rutherford, J.C., Jaron, S., and Winge, D.R. (2003). Aft1p and Aft2p mediate iron-responsive gene expression in yeast through related promoter elements. J Biol Chem *278*, 27636-27643.

Sakuno, T., and Watanabe, Y. (2009). Studies of meiosis disclose distinct roles of cohesion in the core centromere and pericentromeric regions. Chromosome Res *17*, 239-249.

Shakoury-Elizeh, M., Tiedeman, J., Rashford, J., Ferea, T., Demeter, J., Garcia, E., Rolfes, R., Brown, P.O., Botstein, D., and Philpott, C.C. (2004). Transcriptional remodeling in response to iron deprivation in Saccharomyces cerevisiae. Mol Biol Cell *15*, 1233-1243.

Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics *122*, 19-27.

Skibbens, R.V. (2009). Establishment of sister chromatid cohesion. Curr Biol *19*, R1126-1132.

Skibbens, R.V. (2010). Buck the establishment: reinventing sister chromatid cohesion. Trends Cell Biol *20*, 507-513.

Skibbens, R.V., Corson, L.B., Koshland, D., and Hieter, P. (1999). Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes Dev *13*, 307-319.

Sora, S., Lucchini, G., and Magni, G.E. (1982). Meiotic Diploid Progeny and Meiotic Nondisjunction in SACCHAROMYCES CEREVISIAE. Genetics *101*, 17-33.

Stephens, A.D., Haase, J., Vicci, L., Taylor, R.M., 2nd, and Bloom, K. (2011). Cohesin, condensin, and the intramolecular centromere loop together generate the mitotic chromatin spring. J Cell Biol *193*, 1167-1180.

Strom, L., Lindroos, H.B., Shirahige, K., and Sjogren, C. (2004). Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. Mol Cell *16*, 1003-1015.

Sutani, T., Kawaguchi, T., Kanno, R., Itoh, T., and Shirahige, K. (2009). Budding yeast Wpl1(Rad61)-Pds5 complex counteracts sister chromatid cohesion-establishing reaction. Curr Biol *19*, 492-497.

Thompson, S.L., and Compton, D.A. (2011). Chromosomes and cancer cells. Chromosome Res *19*, 433-444.

Tong, A.H., and Boone, C. (2006). Synthetic genetic array analysis in Saccharomyces cerevisiae. Methods Mol Biol *313*, 171-192.

Tong, A.H., Evangelista, M., Parsons, A.B., Xu, H., Bader, G.D., Page, N., Robinson, M., Raghibizadeh, S., Hogue, C.W., Bussey, H., *et al.* (2001). Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science *294*, 2364-2368.

Toth, A., Ciosk, R., Uhlmann, F., Galova, M., Schleiffer, A., and Nasmyth, K. (1999). Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev *13*, 320-333.

Ueta, R., Fujiwara, N., Iwai, K., and Yamaguchi-Iwai, Y. (2007). Mechanism underlying the iron-dependent nuclear export of the iron-responsive transcription factor Aft1p in Saccharomyces cerevisiae. Mol Biol Cell *18*, 2980-2990.

Ueta, R., Fukunaka, A., and Yamaguchi-Iwai, Y. (2003). Pse1p mediates the nuclear import of the iron-responsive transcription factor Aft1p in Saccharomyces cerevisiae. J Biol Chem *278*, 50120-50127.

Uhlmann, F., Wernic, D., Poupart, M.A., Koonin, E.V., and Nasmyth, K. (2000). Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. Cell *103*, 375-386.

Unal, E., Arbel-Eden, A., Sattler, U., Shroff, R., Lichten, M., Haber, J.E., and Koshland, D. (2004). DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. Mol Cell *16*, 991-1002.

Veatch, J.R., McMurray, M.A., Nelson, Z.W., and Gottschling, D.E. (2009). Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. Cell *137*, 1247-1258.

Verdaasdonk, J.S., and Bloom, K. (2011). Centromeres: unique chromatin structures that drive chromosome segregation. Nat Rev Mol Cell Biol *12*, 320-332.

Weber, S.A., Gerton, J.L., Polancic, J.E., DeRisi, J.L., Koshland, D., and Megee, P.C. (2004). The kinetochore is an enhancer of pericentric cohesin binding. PLoS Biol *2*, E260.

Westermann, S., Drubin, D.G., and Barnes, G. (2007). Structures and functions of yeast kinetochore complexes. Annu Rev Biochem *76*, 563-591.

White, M.A., Riles, L., and Cohen, B.A. (2009). A systematic screen for transcriptional regulators of the yeast cell cycle. Genetics *181*, 435-446.

Wong, J., Nakajima, Y., Westermann, S., Shang, C., Kang, J.S., Goodner, C., Houshmand, P., Fields, S., Chan, C.S., Drubin, D., *et al.* (2007). A protein interaction map of the mitotic spindle. Mol Biol Cell *18*, 3800-3809.

Yamaguchi-Iwai, Y., Dancis, A., and Klausner, R.D. (1995). AFT1: a mediator of iron regulated transcriptional control in Saccharomyces cerevisiae. EMBO J *14*, 1231-1239.

Yamaguchi-Iwai, Y., Stearman, R., Dancis, A., and Klausner, R.D. (1996). Iron-regulated DNA binding by the AFT1 protein controls the iron regulon in yeast. EMBO J *15*, 3377-3384.

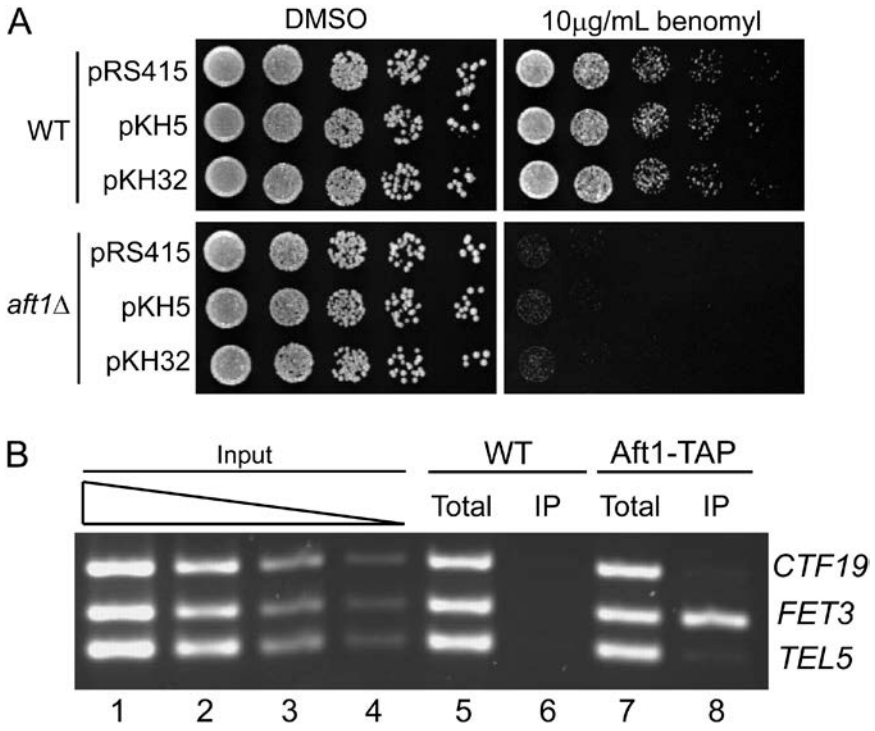
Yamaguchi-Iwai, Y., Ueta, R., Fukunaka, A., and Sasaki, R. (2002). Subcellular localization of Aft1 transcription factor responds to iron status in Saccharomyces cerevisiae. J Biol Chem *277*, 18914-18918.

Yu, H., Braun, P., Yildirim, M.A., Lemmens, I., Venkatesan, K., Sahalie, J., Hirozane-Kishikawa, T., Gebreab, F., Li, N., Simonis, N., *et al.* (2008). High-quality binary protein interaction map of the yeast interactome network. Science *322*, 104-110.

Yuen, K.W., Warren, C.D., Chen, O., Kwok, T., Hieter, P., and Spencer, F.A. (2007). Systematic genome instability screens in yeast and their potential relevance to cancer. Proc Natl Acad Sci U S A *104*, 3925-3930.

Figure 15: *CTF19* does not rescue the benomyl sensitivity of *aft1Δ* cells. (A) Wild type and *aft1Δ* cells transformed with pRS315 (vector control), pKH5 (genomic fragment containing *CTF19*) or pKH32 (HA-tagged *CTF19* fusion clone) were five-fold serially diluted onto YPD plates containing either DMSO or 10ug/mL benomyl. The plates were incubated for 2 days at 30°C. **(B)** Aft1p does not localize to the promoter of *CTF19*. Modified ChIP was performed using an untagged and an Aft1p-TAP strain. Total or Immunoprecipitated (IP) DNA was subjected to multiplex PCR amplification using primers specific to the promoter region of *CTF19*, *FET3* (positive control) and a subtelomeric region of chromosome V (*TEL-V*). The result of this ChIP was representative of three experiments. (Taken from Berthelet et al., 2010)

Appendix A


A
DMSO
10μg/mL benomyl
WT
pRS415
pKH5
pKH32
aft1Δ
pRS415
pKH5
pKH32
B
Input
WT
Aft1-TAP
Total
IP
Total
IP
CTF19
FET3
TEL5
1
2
3
4
5
6
7
8

Appendix B

Table 1: Yeast strains used in this study

Strain	Auxotrophies	Reference or Source
YKB779	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	(Sikorski and Hieter, 1989)
YKB780	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	(Sikorski and Hieter, 1989)
YKB2020	*MATα his3 leu2 ura3 Aft1p-TAP::HIS*	This study
YKB2019	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 Iml3p-Myc::kanMX*	This study
YKB2023	*MATa his3 leu2 ura3 Aft1-TAP::HIS Iml3p-Myc::kanMX*	This study
YKB2285	*MATa leu2 ura3 Aft1-TAP::HIS Iml3p-Myc::kanMX ctf19Δ::HIS3*	This study
YKB2639	*MATa his3 leu2 ura3 Aft1-TAP::HIS Iml3p-Myc::kanMX chl4Δ::TRP1*	This study
YKB2281	*MATa his3 leu2 ura3 Aft1p-TAP::HIS Chl4p-Myc::TRP1*	This study
YKB2284	*MATa his3 leu2 ura3 Chl4p-Myc::TRP1*	This study
YKB2287	*MATa his3 leu2 ura3 Aft1p-TAP::HIS Chl4p-Myc::TRP1 iml3Δ::kanMX*	This study
YKB2640	*MATa his3 leu2 ura3 Aft1-TAP::HIS Chl4p-Myc::TRP1 ctf19Δ::kanMX*	This study
YKB2283	*MATa his3 leu2 ura3 Ctf19p-Myc::TRP1*	This study
YKB2282	*MATα his3 leu2 ura3 Aft1p-TAP::HIS Ctf19p-Myc::TRP1*	This study
YKB2289	*MATa his3 leu2 ura3 Aft1p-TAP::HIS Ctf19p-Myc::TRP1 iml3Δ::kanMX*	This study
YKB2641	*MATα his3 leu2 ura3 Aft1p-TAP::HIS Ctf19p-Myc::TRP1 chl4Δ::kanMX*	This study
YKB2766	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Iml3p-TAP::HIS*	TAP collection
YKB2767	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Iml3p-TAP::HIS aft1Δ::kanMX*	This study
YKB2768	*MATα ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 Ctf19p-Myc::kanMX*	This study
YKB2769	*MATα ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 Chl4p-Myc::kanMX*	This study
YKB2770	*MATα his3 leu2 ura3Iml3-TAP::HIS Ctf19p-Myc::kanMX*	This study

YKB2772	*MATa his3 leu2 ura3 Iml3-TAP::HIS Ctf19p-Myc::kanMX aft1Δ::kanMX*	This study
YKB2777	*MATα ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 trp1-Δ63 Iml3p-3HA::kanMX*	(Pot et al., 2003)
YKB2776	*MATa ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 trp1-Δ63 Iml3p-3HA::kanMX Chl4p-Myc::TRP1*	(Pot et al., 2003)
YKB2902	*MATa ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-Δ1 trp1-Δ63 Iml3p-3HA::kanMX Chl4p-Myc::TRP1 aft1Δ::kanMX*	This study
YKB236	*MATa pep4Δ::LEU2 ura trp ade his Scc1p-HA6::HIS3*	(Toth et al., 1999)
YKB2764	*MATa ura trp ade his Scc1p-HA6::HIS3 aft1Δ::kanMX*	This study
YKB2765	*MATa ura trp his Scc1p-HA6::HIS3 iml3Δ::kanMX*	This study
	Strains used in growth assays:	
YKB1095	*MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 aft1Δ::kanMX*	This study
YKB157	*MATa ura3-52 lys2-801 ade2-101 his3-Δ200 trp1-Δ63 scc1-73*	(Michaelis et al., 1997)
YKB90	*MATa trp1-1 ade2-101 can1-100 leu2-3,112 his3-11,15 ura3 scc2-4*	(Michaelis et al., 1997)
YKB227	*MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 smc3-42*	(Michaelis et al., 1997)
YKB2138	*MATa pep4Δ::G418 ura3-52 leu2-3,112 his3-11,15 bar1 GAL+ eco1-203*	(Skibbens et al., 1999)
YKB2274	*MATα his3 leu2 ura3 iml3Δ::kanMX*	This study
YKB2749	*MATα ura3-52 lys2-801 ade2-101 his3-Δ200 trp1-Δ63 scc1-73 aft1Δ::kanMX*	This study
YKB2752	*MATα his3 ura3 scc1-73 iml3Δ::kanMX*	This study
YKB2753	*MATα trp ade2-101 leu2 his3 ura3 scc2-4 aft1Δ::kanMX*	This study
YKB2755	*MATα his3 leu2 ura3 scc2-4 iml3Δ::kanMX*	This study
YKB2757	*MATα ade2 trp1 leu2 his3 ura3 smc3-42 aft1Δ::kanMX*	This study
YKB2759	*MATα his3 leu2 ura3 smc3-42 iml3Δ::kanMX*	This study
YKB2761	*MATα ura3-52 leu2 his3 eco1-203 aft1Δ::kanMX*	This study
YKB2762	*MATα his3 leu2 ura3 eco1-203 iml3Δ::KanMX*	This study
	Strains used in mitotic cohesion assay:	
YKB2635	*MATa ura3 trp1 leu2 his3-11 MET-CDC20::URA3*	(Fernius and

	promURA3::tetR::GFP::LEU2, cenIV::tetOx448::URA3	Marston, 2009)
YKB2636	*MATa ura3 trp1 leu2 his3-11 MET-CDC20::URA3 promURA3::tetR::GFP::LEU2, cenIV::tetOx448::URA3 iml3Δ::kanMX*	(Fernius and Marston, 2009)
YKB2637	*MATa ura3 trp1 leu2 his3-11 MET-CDC20::URA3 promURA3::tetR::GFP::LEU2, cenIV::tetOx448::URA3 aft1Δ::kanMX*	This study
YKB2638	*MATa ura3 trp1 leu2 his3-11 MET-CDC20::URA3 promURA3::tetR::GFP::LEU2, cenIV::tetOx448::URA3 iml3Δ::kanMX6 aft1Δ::TRP1*	This study
YKB2903	*MATa ura3 trp1 leu2 his3-11 MET-CDC20::URA3 promURA3::tetR::GFP::LEU2, cenIV::tetOx448::URA3 aft1-TADΔ-Myc::TRP1*	This study
	Strains used in meiotic cohesion assay:	
YKB2589	*MATa/α ho::LYS2 (lys2?) ura3 leu2 his4X trp1::hisG promURA3-TetR-GFP::LEU2 TETOx224-URA3*	(Fernius and Marston, 2009)
YKB2590	*MATa/α ho::LYS2 (lys2?) ura3 leu2 his4X trp1::hisG promURA3-TetR-GFP::LEU2 TETOx224-URA3 aft1Δ::kanMX*	This study

Akil Hamza

Education:

Master of Science, Biochemistry
University of Ottawa (2009-2011)
Thesis Supervisor: Dr. Kristin Baetz

Honours Bachelor of Science with Specialization in Biochemistry and Minor in Psychology
University of Ottawa (2004-2009)
CGPA: 8.4 Magna Cum Laude

Awards/Recognition:

- (OGS) Ontario Graduate Scholarship (2011-2012) - $15000 - Declined
- University of Ottawa Excellence Scholarship (2010-2011) - $6250
- (CIHR) Canadian Institutes of Health Research - Masters' Award (2010-2011) - $17500
- (OGS) Ontario Graduate Scholarship (2010-2011) - $15000 - Declined
- International Yeast Genetics and Molecular Biology Meeting Travel Grant (University of British Columbia) (2010) -$1000
- University of Ottawa Admission Scholarship Sciences M.Sc. (2009-2010) - $6250
- University of Ottawa Merit Scholarship (2008) - $1000
- University of Ottawa Admission Scholarship Sciences B.Sc. (2004-2008) - $6000
- Deans Honour List - Faculty of Science (2004-2005; 2007-2008; 2008-2009)

Publications:

- **Hamza A**, Baetz, K. The iron-responsive transcription factor Aft1 interacts with the kinetochore protein Iml3 and promotes pericentromeric cohesin. J Biol Chem. 2011 Dec 8. [Epub ahead of print]

- Berthelet S, Usher J, Shulist K, **Hamza A**, Maltez N, Johnston A, Fong Y, Harris LJ, Baetz K. (2010) Functional Genomics Analysis of the Saccharomyces cerevisiae Iron Responsive Transcription Factor Aft1 Reveals Iron-independent Functions. Genetics 185(3):1111-28

- Gupta P, Bilinska ZT, Sylvius N, Boudreau E, Veinot JP, Labib S, Bolongo PM, **Hamza A**, Jackson T, Ploski R, Walski M, Grzybowski J, Walczak E, Religa G, Fidzianska A, Tesson F. (2010) Genetic and ultrastructural studies in dilated cardiomyopathy patients: a large deletion in the lamin A/C gene is associated with cardiomyocyte nuclear envelope disruption. Basic Res Cardiol. 105(3):365-77

Research/Laboratory Experience:

- (September 2009-present) Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Ontario, Canada (M.Sc.)
  Principal Investigator: Dr. Kristin Baetz
  Project: Deciphering the role of Aft1p in chromosome stability

- (May 2007-April 2008) Lab of Genetics of Cardiac Diseases, Ottawa Heart Institute, University of Ottawa, Ottawa, Ontario, Canada (B.Sc.)
  Principal Investigator: Dr. Frederique Tesson
  Project: Genetic causes of Dilated Cardiomyopathy

Academic Employment:

- (January-April 2011) Teaching Assistant - BCH2333 Biochemistry Laboratory, University of Ottawa
  Supervised, coordinated, and guided students to complete laboratory experiments. Instructed and mentored students to write lab reports and evaluated their reports.

Conferences:

- Ottawa Institute Of Systems Biology Symposium - Montebello, Quebec (June 2011) - Poster Presentation
  Presentation Title: Deciphering the role of Aft1p in chromosome stability

- International Yeast Genetics and Molecular Biology Meeting - University of British Columbia, Vancouver, Canada (July 2010) - Poster Presentation
  Presentation Title: Deciphering the role of Aft1p in chromosome stability