Iron-responsive Transcription Factor Aft1 Interacts with Kinetochore Protein Iml3 and Promotes Pericentromeric Cohesin*

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Background: The budding yeast transcription factor Aft1 has been implicated in chromosome stability.

Results: Aft1 interacts with the kinetochore protein Iml3 and is required for promoting pericentromeric cohesin.

Conclusion: We have identified a new factor impacting the pericentric chromatin spring.

Significance: This discovery explains why Aft1 mutants display chromosome segregation defects and sheds light on how the Ctf19 complex promotes pericentric cohesin.

The Saccharomyces cerevisiae iron-responsive transcription factor, Aft1, has a well established role in regulating iron homeostasis through the transcriptional induction of iron-regulon genes. However, recent studies have implicated Aft1 in other cellular processes independent of iron regulation such as chromosome stability. In addition, chromosome spreads and two-hybrid data suggest that Aft1 interacts with and co-localizes with kinetochore proteins; however, the cellular implications of this have not been established. Here, we demonstrate that Aft1 associates with the kinetochore complex through Iml3. Furthermore, like Iml3, Aft1 is required for the increased association of cohesin with pericentric chromatin, which is required to resist microtubule tension, and aft1Δ cells display chromosome segregation defects in meiosis. Our work defines a new role for Aft1 in chromosome stability and transmission.

In the budding yeast, Saccharomyces cerevisiae, iron levels are regulated by the transcription factor Aft1 (reviewed in Ref. 1). Iron depletion activates Aft1 to induce the expression of ~25 genes referred to as the “iron-regulon” by binding the consensus sequence 5′-YRCACCCR-3′ present in iron-responsive elements of these target genes (2, 3). 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 Aft1 has an established role as an iron-responsive transcription factor, many studies have implicated Aft1 in other cellular functions, including chromosome stability, cell cycle regulation, DNA damage, cell wall stability, and mitochondrial function (4–10). Evidence implicating Aft1 in chromosome stability first originated from synthetic genetic array studies that determined aft1Δ mutants could not tolerate either over-expression or loss-of-function of kinetochore genes (6). Moreover, chromosome transmission fidelity assays determined that aft1Δ mutant cells have defects in maintaining artificial chromosome fragments (4, 6, 7), and aft1Δ cells were shown to be hypersensitive to benomyl treatment (4). Benomyl is a microtubule-destabilizing drug, and many mutants with defects in chromosome stability share the same sensitivity (11). In contrast to most chemical sensitivities and phenotypes of aft1Δ cells, neither the benomyl sensitivity nor chromosome transmission fidelity defects of aft1Δ mutant cells can be rescued by increased extracellular iron in media (4). Furthermore, the paralog of AFT1, AFT2, and other iron-regulon genes were not identified in the genome-wide studies that implicated Aft1 in chromosome stability (4, 6, 7) nor are aft2Δ mutant cells sensitive to benomyl treatment (4). Overall, this suggests that the role of Aft1 in chromosome stability is not related to regulation of the iron-regulon or iron homeostasis.

Many lines of evidence suggest that Aft1 has a role at the kinetochore. The kinetochore complex consists of many multiprotein subcomplexes that assemble in a hierarchical fashion onto centromeric (CEN) DNA physically linking the chromosomes to microtubule spindles (reviewed in Ref. 12). Aft1 was found to co-localize with kinetochore proteins using chromosome spreads (6), and Aft1 has been shown to interact with the kinetochore protein Iml3 by yeast-two hybrid assays (13, 14). Iml3 is a peripheral member of the Ctf19 complex that is composed of the COMA subcomplex (Ctf19, Okp1, Mcm21, and Amel) along with Chl4 and Iml3 (15, 16). Studies investigating the hierarchical dependences of the kinetochore proteins have established that Iml3 localization to the kinetochore is dependent on its interacting partner, Chl4, which in turn is dependent on Ctf19 (Fig. 1A) (15). Although very little is known about Iml3 or associated Ctf19 complex function, it has been shown that these proteins are required for the increased association of cohesin (reviewed in Refs. 17, 18) at the pericentromere and centromere regions in both mitosis and meiosis (5, 19–21). Contrary to its role in embracing sister chromatids at the arms, cohesin, along with condensin, functions at pericentric DNA to form an intramolecular loop with the centromere as its apex.

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(22). This establishes a pericentromeric chromatin spring-like structure that counterbalances microtubule forces during metaphase (23) and ensures sister chromatin bi-orientation (reviewed in 24). Despite the importance of pericentric cohesin, the molecular mechanism by which the Ctf19 complex recruits cohesin has yet to be ascertained.

In this study we further investigate the role of Aft1 in chromosome stability and its interaction with the kinetochore. Surprisingly, we discover that Aft1 interacts with Im3 and has a role in promoting pericentric cohesin.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains**—Yeast strains used in this study are listed in Table 1. Deletion strains and epothe tag integrations made for this study were designed using a standard PCR-mediated gene insertion technique (25) and confirmed by PCR analysis.

**Whole-cell Extract Co-immunoprecipitation**—Cells were grown in 200 ml of YPD at 30 °C to mid-log phase (A600 ~0.6–0.8) and harvested. Cell pellets were resuspended in Tackett extraction buffer (20 mM HEPEs, pH 7.4, 0.1% Tween 20, 2 mM MgCl2, 200 mM NaCl, and protease inhibitors (Sigma, P-8215)) and an equal volume of glass beads (BioSpec Products, 11079105). Cells were lysed through vortexing (five 1-min blasts with incubation on ice between vortexing) followed by centrifugation at 3000 rpm for 15 min to isolate whole-cell extract. Samples were normalized by protein concentration and incubated end-over-end for 2 h at 4 °C with magnetic Dynabeads (Dynal, Invitrogen, 140-01) crosslinked to rabbit immunoglobulin G (IgG) (Chemicon, PP64) magnetic Dynabeads (Dynal, Invitrogen, 143-01) crosslinked to rabbit immunoglobulin G (IgG) (Chemicon, PP64) or with magnetic protein G Dynabeads (Invitrogen, 100.03D) crosslinked to mouse HA.11 monoclonal antibody (Covance, MMS-101P). Dynabeads were collected with a magnet, washed three times with 1 ml of cold Tackett extraction buffer, and resuspended in 25 μl of modified 1× loading buffer (50 mM Tris, pH 6.8, 2% SDS, 0.1% bromphenol blue, 10% glycerol). Purified and co-purified proteins were eluted from the beads at 65 °C for 10 min and transferred to a new tube. β-Mercaptoethanol was added to a final concentration of 200 mM, and samples were placed at 95 °C for 10 min. Immunoprecipitates were subjected to SDS-PAGE and Western blot-
ting. Primary antibodies used are as follows: anti-TAP (Thermo Scientific, CAB1001, 1:5000), anti-Myc (Roche Applied Science, 1166714901, 1:800), and anti-HA (Covance, MMS-101P, 1:1000). Secondary antibodies were HRP-linked, goat anti-rabbit IgG (Chemicon, AP307P, 1:5000), and goat anti-mouse IgG (Bio-Rad, 170 – 6516, 1:5000).

Dot Assays and Growth Conditions—Yeast strains were grown in YPD at 25 °C to mid-log phase and then spotted in 10-fold serial dilutions (A_{600} = 0.1, 0.01, 0.001, and 0.0001) onto YPD plates and incubated at 25, 30, and 33 °C for 2 days before epic-white imaging using the Molecular Image ChemiDoc XRS System (Bio-Rad). Dot assay experiments were repeated in triplicate using different isolates of each strain.

Chromatin Immunoprecipitation (ChIP)-Multiplex PCR and qPCR—ChIP was carried out as described previously (15). Immunoprecipitated DNA was amplified using multiplex-PCR with the following primer pairs: CEN3 forward (5’-GGCATCACGGCCTAACATATGG) and CEN3 reverse (5’-GAGCAAAAATTTTACCCAGTAAAGC) were obtained from Ref. 26; FET3 forward (5’-GGTCCCTCACTAGCTGAGG) and FET3 reverse (5’-GGATCGACTGGTGGTGTGATCC). PCR products were resolved on a 3% agarose gel and visualized with ethidium bromide. For qPCR-ChIP, cells were grown at 25 °C to mid-log phase and arrested as described previously (19). qPCR correlation using the Bio-Rad MiniOpticon real time PCR system. ChIP enrichment/input values were calculated as described previously (19). Primer sequences were obtained from Ref. 19, and the pericentromere, centromere, and arm primers correspond to primer pairs P2, C1, and A3, respectively. The primer sequences used are as follows: pericentromere forward (5’-ATTGTTTAGAAACGGGAACA) and reverse (5’-GTTCACCTTCTGCACTTCC); centromere forward (5’-ACACGAGCGAATAATGTAAC) and reverse (5’-TGATTTAAGCATGTACCTTTT); and arm forward (5’-GAAAGCGCAACAGCTAGATTA) and reverse (5’-CAACGCTTATACACCAAG).

Cohesion Assays and Microscopy—The mitotic cohesion assay was performed as described previously (19). For the mitotic cohesion assay, sporulation was performed at 30 °C as described previously (27). Cells for both assays were fixed as described previously (28). Microscopy was performed on a Leica DMi600B fluorescent microscope (Leica Microsystems GmbH, Wetzler Germany), equipped with a Sutter DG4 light source (Sutter Instruments), Luvdi emission filter wheel with Chroma band pass emission filters (Ludl Electronic Products Ltd.,) and Hamamatsu Orca AG camera (Hamamatsu Photonics, Herrsching am Ammersee, Germany). Images were collected and analyzed using Velocity 4.3.2 Build 23 (PerkinElmer Life Sciences). Analysis was performed on images collapsed into two dimensions using the “extended focus” in Velocity.

RESULTS

Aft1 Interacts with the Kinetochore Protein Iml3—To elucidate the function of Aft1 in chromosome stability, we first sought to identify the individual kinetochore protein or protein subcomplex that interacts with Aft1. As Iml3 was found to interact with Aft1 by yeast two-hybrid (13, 14), we decided to confirm this physical interaction by a secondary method. Co-immunoprecipitation experiments with strains containing TAP-tagged Aft1 and Myc-tagged Iml3, Chl4, or Ctf19 confirmed that Aft1-TAP can co-purify proteins of the Ctf19 subcomplex (Fig. 1, B–D, lanes 4).

To further dissect the Aft1-kinetochore interaction, we systematically created deletion mutant strains based on the established hierarchical dependences of the Ctf19 complex (Fig. 1A) (15). We first investigated whether the Aft1-Ctf19 interaction (Fig. 1B, lane 4) is dependent on Chl4 or Iml3. In a�Δ background that abolishes the Iml3-Ctf19 interaction (15), the Aft1-Ctf19 interaction was disrupted (Fig. 1B, lane 5) indicating that this interaction was either through Chl4 or Iml3. In an iml3Δ background that maintains the Chl4-Ctf19 interaction, albeit in a reduced fashion (15, 19), the Aft1-Ctf19 interaction was also disrupted (Fig. 1B, lane 6). These results suggest that Aft1 is co-purifying Chl4 and Ctf19 through Iml3. To confirm this, we next examined the effect of ctf19Δ and iml3Δ mutations on the Aft1-Chl4 interaction (Fig. 1C, lane 4). In a ctf19Δ background that maintains the Iml3-Chl4 complex (15), the Aft1-Chl4 interaction was preserved (Fig. 1C, lane 6). These results confirmed the findings of the previous two experiments, as we show that in both ctf19Δ and chl4Δ mutants background Aft1 can still co-immunoprecipitate Iml3 (Fig. 1D, lanes 5 and 6). Therefore, our results confirmed the Aft1-Iml3 two-hybrid interaction and indicated that Aft1 is interacting with kinetochore COMA complex subunits through Iml3.

Iml3 Kinetochore Localization Is Not Dependent on Aft1—To determine whether Aft1 impacts the localization of Iml3 to the CEN, we performed a chromatin immunoprecipitation (ChIP) assay using an untagged control, Iml3-TAP, and Iml3-TAP aft1Δ strains (Fig. 2A). TAP-tagged Iml3 was immunoprecipitated from formaldehyde cross-linked whole-cell extracts, and co-precipitated DNA was subjected to multiplex PCR with primers specific to the centromere of chromosome 3 (CEN3) and the promoter of FET3 (negative control). Our results indicate that Aft1 does not impact the centromeric localization of Iml3 (Fig. 2B). To further confirm our results, we carried out protein co-immunoprecipitation experiments to investigate if Iml3-Ctf19 and Iml3-Chl4 interactions are dependent on Aft1. Our results demonstrate that the Iml3-Ctf19 and Iml3-Chl4 interactions are not disrupted in an aft1Δ background (Fig. 2, C and D). Taken together, our results indicate that Aft1 is not regulating Iml3 localization to CENs or interaction with COMA proteins.

aft1Δ and iml3Δ Display Similar Synthetic Genetic Interactions with Cohesion Mutants—Given that Iml3 has been implicated to function in the cohesion pathway (5, 19, 29, 30) and that genetic interactions predict functional relationships (31), we wanted to determine whether aft1Δ and iml3Δ mutants display synthetic genetic interactions with temperature-sensitive
mutants of the sister chromatic cohesion pathway: *scc1-73, scc2-4, smc3-42* (32), and *eco1-203* (33). Scc1 and Smc3 are part of the tripartite cohesin ring that physically links sister chroma-

**FIGURE 2. Aft1 does not impact the kinetochore localization and protein interactions of Iml3.** A, presence or absence of Aft1 does not impact the purification of Iml3-TAP. Western blot analysis of Iml3-TAP purified by ChIP (IP) and whole-cell extract (WCE). B, Iml3 localization to the centromere is not affected by Aft1. Traditional ChIP performed using untagged, Iml3-TAP, and Iml3-TAP/aft1/H9004 strains. Total or immunoprecipitated DNA was subjected to multiplex PCR amplification using primers specific to the centromere of chromosome 3 (*CEN3*) and the negative control *FET3*. C, Aft1 co-purification with the kinetochore protein Chl4 is dependent on Iml3 but not Ctf19. Anti-TAP immunoprecipitations were performed with strains containing different combinations of TAP-tagged Aft1 and Myc-tagged Chl4 in the presence or absence of Ctf19 and Iml3. D, Aft1 co-purification with the kinetochore protein Iml3 is independent of Ctf19 and Chl4. Anti-TAP immunoprecipitations were performed with strains containing different combinations of TAP-tagged Aft1 and Myc-tagged Iml3 in the presence or absence of Ctf19 and Chl4. For all immunoprecipitations, 10% of the eluate was loaded in the TAP blot, and 90% of the eluate was loaded in the Myc blot. IP, immunoprecipitations; WCE, whole-cell extract.
(reviewed in Ref. 18). As expected, we found that iml3Δ mutants display negative genetic interactions with all the temperature-sensitive cohesion mutants at 25 °C. Similarly, we 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 was not as pronounced as with iml3Δ (Fig. 3). This suggests that like Iml3, Aft1 may have a role in sister chromatid cohesion.

**Defective Mitotic Pericentromeric Cohesion in aft1Δ Mutants**—It has been shown previously that Iml3 and other Ctf19 complex proteins exhibit defects in resisting microtubule pulling forces (19) and increased spindle length in metaphase (23). To determine whether Aft1 displays similar defects, we used strains that express TetR-GFP and have tetO arrays integrated 2.4 kb from the CEN of chromosome 4 (+2.4CEN4-GFP), and we asked if sister chromatids are more frequently separated in a metaphase arrest (Fig. 4A). Wild-type, iml3Δ, aft1Δ, and aft1Δiml3Δ cells under the control of a methionine-repressible promoter (MET-CDC20) were released from a G1 α-factor block into methionine-rich media. This induces a metaphase arrest through depletion of Cdc20 (required for the metaphase to anaphase transition) (34). Sister chromatids in this metaphase arrest remain under tension from the microtubule pulling forces (Fig. 4A), and the separation of sister pericentric regions were scored by counting the number of cells that have 1 or 2 GFP foci (Fig. 4B). Consistent with previous studies (19), our results show that ~30% of wild-type cells exhibit GFP dot separation, and this increases to 60% in iml3Δ cells that exhibit defects in counter-balancing spindle forces (Fig. 4C). Approximately 55% of aft1Δ cells have separated GFP foci indicating that Aft1, like Iml3, is required for establishing pericentric cohesion in mitosis. As aft1Δiml3Δ cells did not display additive defects, it suggests that Aft1 and Iml3 function together to establish pericentric chromatin that can balance mitotic forces.

We were then interested to determine which domain of Aft1 is responsible for this phenotype. Previous studies have mapped the transactivation domain of Aft1 to the C-terminal region and within amino acids 413–690 (35), and the N-terminal region contains the DNA binding domain (36). As such, we deleted the transactivation domain of Aft1, or amino acids 413–690, to create the truncated aft1-TADΔ strain (Fig. 4D) and asked whether we observed the same phenotype as aft1Δ cells. Our results show that the aft1-TADΔ strain displays defects in resisting microtubule tension similar to aft1Δ cells (Fig. 4C) indicating that the transactivation domain of Aft1 is contributing to regulate spindle length dynamics.
**Aft1 Is Required for Pericentromeric Cohesion**

**A**

**Meiosis I**

**Meiosis II**

**B**

**Legend**

- **% cells**
- **WT**
- **aft1A**

**FIGURE 5. Aft1 is required for proper chromosome segregation during meiosis.** A, schematic showing the segregation of chromosome 5 labeled with GFP. Sister chromatid cohesion is represented by black lines between sister chromatids. GFP is represented by black dot. B, aft1Δ cells display meiotic chromosome segregation defects. Wild-type (WT) and aft1Δ diploids that have GFP-labeled chromosome 5 (tetR-GFP::LEU2 URA3::tetOx224) at URA3 (38.4 kb from the centromere) were induced to sporulate at 30°C. 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.

**DISCUSSION**

Several genome-wide studies identified Aft1 as a potential regulator in chromosome stability (4, 5, 7, 13, 14). In this study, we demonstrate that Aft1 interacts with kinetochore proteins Ctf19 and Chl4 through Iml3, and like these members of the Ctf19 complex, we found that Aft1 contributes to the enrichment of cohesin at the pericentromere. The mechanism by which Iml3 and other Ctf19 complex members promote cohesion enhancement at the centromere is likely the key protein controlling this process. The pericentromeric separation of mitotic sister chromatids displayed by aft1Δiml3Δ mutant cells is comparable with the single mutants (Fig. 4C). As Aft1 does not interact the impact of Iml3 with the kinetochore (Fig. 2), it suggests that one role Iml3 may be playing is to recruit Aft1 to the kinetochore, which in turn aids in the recruitment of pericentric cohesion. However, as the strength of the genetic interactions of aft1Δ mutants with the cohesion pathway mutants is not as pronounced as iml3Δ mutants (Fig. 3), it suggests that Iml3 plays additional roles in the cohesion pathway not shared by Aft1.

Although Aft1 has not been linked to the transcription of genes implicated in chromosome stability or benomyl resistance (4), we cannot eliminate the possibility that part of the
role Aft1 plays in chromosome stability is mediated through transcription. With that said, the physical interaction between Aft1 and Iml3 (Fig. 1) (13, 14) and the co-localization of Aft1 with kinetochore proteins (6) strongly argues for a role for Aft1 directly at the kinetochore. Aft1 has not been identified in the numerous proteomic studies of the kinetochore (12, 16) but only via two-hybrid screens (13, 14) suggesting that Aft1 interaction with the kinetochore may be transient (38). Indeed, we may have been able to detect the Aft1-Iml3 interaction because our purifications utilized low speed centrifugation, which has been shown to increase the solubility of chromatin-associated proteins (39).

What are the possible roles for Aft1 at the kinetochore? One possibility is that Aft1 is physically interacting with and recruiting members of the cohesin complex or the Scc2-Scc4 loader complex to the centromere; however, like Iml3, no physical interaction between Aft1 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 to the centromere where they specifically position or add epigenetic marks to pericentromeric histones which in turn recruit Scc2-Scc4. 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 (40, 41). Indeed, histone modifications dependent on a functional kinetochore have been reported at the centromere, and these modifications contribute to chromosome segregation (42).

Also, establishment of cohesion has been linked to numerous chromatin regulators and histone modifications (43–47), and chromatin-remodeling complexes were also found to interact with kinetochore proteins and localize to the centromere (13, 48, 49). Could Aft1 be recruiting such factors to the centromere? The transactivation domain of Aft1, which is believed to recruit chromatin remodelers and modifiers to iron-regulon promoters (35), is required for resisting microtubule pulling forces (Fig. 4C). Furthermore, affinity-capture mass spectrometry studies have demonstrated Aft1 co-purifying with protein subunits of the SWI/SNF chromatin remodeler (39) and the lysine acetyltransferases SAGA (50) and NuA4 (39); all three have been implicated in chromosomal stability and/or cohesion defects (5, 26, 49). Exactly how Aft1 contributes to the structure and function of the pericentric chromatin spring will require additional study. However, as numerous other transcription factors have been shown to interact with kinetochore proteins (13), the role of transcription factors in pericentromeric chromatin may not be limited to Aft1 but rather to a general mechanism that aids in the establishment of the unique chromatin environment of the pericentromere.

FIGURE 6. Levels of Scc1–6HA are reduced at the pericentromere and centromere in aft1Δ mutants. A, presence or absence of Aft1 does not impact the amount of Scc1–6HA purified by ChIP. Western blot analysis of Scc1–6HA purified by ChIP (IP) and whole-cell extract (WCE) in wild-type, aft1Δ, and iml3Δ mutants. B, analysis of Scc1–6HA association in cells arrested in metaphase of mitosis in the absence of microtubules. Wild-type (no tag), SCC1–6HA, iml3Δ SCC1–6HA, and aft1Δ SCC1–6HA cells were arrested in metaphase in the presence of microtubule-depolymerizing drugs nocodazole and benomyl. Metaphase arrest was confirmed by bud count. qPCR analysis of Scc1–6HA levels were performed at three different regions of chromosome 4, and results are the mean of three independent experiments; error bar indicates 1 S.D.
Aft1 Is Required for Pericentromeric Cohesion

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REFERENCES

1. Philpott, C. C., and Prothenko, O. (2008) Response to iron deprivation in Saccharomyces cerevisiae. Eukaryot. Cell 7, 20–27
2. 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
3. Yamaguchi-Iwai, Y., Stearn, R., Dancis, A., and Klausner, R. D. (1996) Iron-regulated DNA binding by the AFT1 protein controls the iron regulation in yeast. EMBO J. 15, 3377–3384
4. Berthelet, S., Shulist, K., Hamza, A., Maltez, N., Johnston, A., Fields, S., Chan, C. S., Drubin, D., Barnes, G., and Baetz, K. (2010) Functional genomics analysis of the Saccharomyces cerevisiae iron-responsive transcription factor Aft1 reveals iron-independent functions. Genetics 185, 1111–1128
5. 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
6. Measday, V., Baetz, K., Guzzo, J., Yuen, K., Kwok, T., Sheik, B., Ding, H., Ueta, R., Hoac, T., Cheng, B., Pot, I., Tong, A., Yamaguchi-Iwai, Y., Boone, C., Hieter, P., and Andrews, B. (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
7. 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
8. Lee, W., St, Onge, R. P., Proctor, M., Flaherty, P., Jordan, M. I., Emili, A., Buratowski, S., Hieter, P., and Greenblatt, J. F. (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, 13153–13158
9. 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
10. Klein, F., Marhr, P., Galova, M., Boucomo, 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
11. Ghosh, S. K., Sow, 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
12. 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
13. Tong, A. H., Evangelista, M., Parsons, A. B., Xu, H., Bader, G. D., Pagé, N., Robinson, M., Raghuzibadeh, S., Hogue, C. W., Bussey, H., Andrews, B., Tyers, M., and Boone, C. (2001) Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294, 2364–2368
14. Michaelis, C., Ciosk, R., and Nasmyth, K. (1997) Cohesins. Chromosomal proteins that prevent premature separation of sister chromatids. Cell 91, 35–45
15. 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
16. Uhlmann, F., Wernic, D., Poupard, 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
17. 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
18. Yamaguchi-Iwai, Y., Dancis, A., and Klausner, R. D. (1995) AFT1. A member of the Cae1p protein family that prevents premature separation of sister chromatids during meiosis in Saccharomyces cerevisiae. Genetics 141, 1167–1180
19. 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
20. 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
21. Eckert, C. A., Gradvall, 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
22. Ye, H., Haase, J., Paliulis, L. V., Joglerk, A., Bond, L., Bouck, D., Salmon, E. D., and Bloom, K. S. (2008) Pericentromeric cohesion is organized into an intramolecular loop in mitosis. Curr. Biol. 18, 81–90
23. Stephens, A. D., Haase, J., Vicci, L., Taylor, R. M., 2nd, and Bloom, K. (2011) Cohesins, condensin, and the intramolecular centromere loop together generate the mitotic kinetochore spring. J. Cell Biol. 193, 1167–1180
24. Verdaasdonk, J. S., and Bloom, K. (2011) Centromeres. Unique chromatin structures that drive chromosome segregation. Nat. Rev. Mol. Cell Biol. 12, 320–332
25. Longtine, M. S., McKenzie, A., 3rd, Demarini, D. J., Shah, N. G., Wach, A., Brachot, A., Philippson, 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
26. Krogan, N. J., Baetz, K., Keogh, M. C., Datta, N., Sawa, C., Kwock, T. C., Thompson, N. J., Davey, M. G., Pootoolal, J., Hughes, T. R., Emili, A., Buratowski, S., Hieter, P., and Greenblatt, J. F. (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
27. 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
28. Philpott, C. C., Rashford, J., Yamaguchi-Iwai, Y., Rosella, 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, 3026–3036
29. Tan, A. H., Evangelista, M., Parsons, A. B., Xu, H., Bader, G. D., Pagé, N., Robinson, M., Raghuzibadeh, S., Hogue, C. W., Bussey, H., Andrews, B., Tyers, M., and Boone, C. (2001) Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294, 2364–2368
30. Michaelis, C., Ciosk, R., and Nasmyth, K. (1997) Cohesins. Chromosomal proteins that prevent premature separation of sister chromatids. Cell 91, 35–45
31. 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
32. Uhlmann, F., Wernic, D., Poupard, 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
33. 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
34. Baetz laboratory for critical reading of the manuscript.
2763–2788

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

40. 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

41. Ström, L., Lindroos, H. B., Shirahige, K., and Sjögren, C. (2004) Postreplicative recruitment of cohesin to double strand breaks is required for DNA repair. Mol. Cell 16, 1003–1015

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

43. 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

44. 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 remodeling complex that loads cohesin onto human chromosomes. Nature 418, 994–998

45. 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

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

47. Oum, J. H., Seong, C., Kwon, Y., Ji, J. H., Sid, A., Ramakrishnan, S., Ira, G., Malkova, A., Sung, P., Lee, S. E., and Shim, E. Y. (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

48. 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

49. Gikopoulos, 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

50. 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

51. 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

52. Tóth, 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