Aurora controls sister kinetochore mono-orientation and homolog bi-orientation in meiosis-I

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Aurora-B kinases are important regulators of mitotic chromosome segregation, where they are required for the faithful bi-orientation of sister chromatids. In contrast to mitosis, sister chromatids have to be oriented toward the same spindle pole in meiosis-I, while homologous chromosomes are bi-oriented. We find that the fission yeast Aurora kinase Ark1 is required for the faithful bi-orientation of sister chromatids in mitosis and of homologous chromosomes in meiosis-I. Unexpectedly, Ark1 is also necessary for the faithful mono-orientation of sister chromatids in meiosis-I, even though the canonical mono-orientation pathway, which depends on Moa1 and Rec8, seems intact. Our data suggest that Ark1 prevents unified sister kinetochores during metaphase-I from merotelic attachment to both spindle poles and thus from being torn apart during anaphase-I, revealing a novel mechanism promoting monopolar attachment. Furthermore, our results provide an explanation for the previously enigmatic observation that fission yeast Shugoshin Sgo2, which assists in loading Aurora to centromeres, and its regulator Bub1 are required for the mono-orientation of sister chromatids in meiosis-I.

The EMBO Journal (2007) 26, 4475–4486. doi:10.1038/sj.emboj.7601880; Published online 11 October 2007

Subject Categories: cell cycle
Keywords: aurora; meiosis; mitosis

Introduction

For eukaryotic chromosome segregation to occur correctly, the two copies of the chromosome (sister chromatids) need to be physically connected. This is accomplished by the ‘cohesin’ complex (Haering and Nasmyth, 2003), which links sister chromatids from their generation in S-phase until the onset of anaphase. By metaphase, the two sister chromatids become attached to microtubules emanating from opposite spindle poles (bi-orientation). A central component ensuring the bi-orientation of sister kinetochores is the ‘chromosomal passenger complex’ (CPC) that is composed of Aurora-B, INCENP, Borealin/Dasra and Survivin (Vagnarelli and Earnshaw, 2004). In anaphase, cohesion between the sister chromatids is released, which is accomplished by cleavage of the Scc1/Rad21 subunit of cohesin by the protease separase (Uhmann, 2003), a process that is under control of the mitotic spindle checkpoint (Musacchio and Salmon, 2007). Similar principles govern meiotic chromosome segregation (Lee and Orr-Weaver, 2003; Watanabe, 2004), but in contrast to mitosis, two rounds of chromosome segregation follow only one round of DNA replication in order to generate haploid gametes. Several modifications allow this two-step process (Petronczki et al., 2003): homologous chromosomes become connected via chiasmata, which result from crossover recombination during meiotic prophase, and sister kinetochores on each chromosome adopt a side-by-side rather than back-to-back conformation (Goldstein, 1981). These two mechanisms allow the recognition of homologous chromosomes as entities destined for opposite poles during anaphase-I. In addition, only cohesion between chromosome arms is lost during anaphase-I, which allows the separation of homologs; cohesion at the centromere is preserved depending on Shugoshin proteins (Watanabe, 2005), and sister chromatids therefore stay connected so that they can be properly segregated during meiosis-II. For meiosis-II, kinetochores are again in a back-to-back position, and chromosome segregation is very similar to mitosis.

In all model eukaryotes that have been studied, Aurora-B kinases are required for the proper bi-orientation of sister chromatids in mitosis. In the absence of Aurora-B, syntelic (both sister kinetochores attached to the same spindle pole) or merotelic (one kinetochore attached to two opposing spindle poles) attachment of chromosomes occurs with increased frequency (Tanaka et al., 2002; Hauf et al., 2003; Cimini et al., 2006; Knowlton et al., 2006). These malattachments escape the surveillance by the mitotic spindle checkpoint, indicating that Aurora-B kinases are also required for proper checkpoint function. Budding and fission yeast have a single Aurora kinase, Ipl1 and Ark1, respectively. These single Aurora kinases are thought to be homologous to Aurora-B and presumably interact with INCENP (Schizosaccharomyces pombe S.p. Pic1) and Survivin (S.p. Bir1) homologs (Kim et al., 1999; Leveryson et al., 2002; Vanoosthuyse et al., 2007). Whereas Ipl1 has been shown to be required for the proper bi-orientation of chromosomes (Francisco and Chan, 1994; Biggins et al., 1999; Tanaka et al., 2002), a role for Ark1 in regulating chromosome attachment has not been demonstrated. Fission yeast cells that lack Ark1...
fail to divide the chromatin during anaphase, but nevertheless proceed to seption, resulting in a cut ('cell untimely torn') phenotype (Petersen and Hagan, 2003). Mutants in the condensin complex, which is required for compaction of chromatin during mitosis, display a similar phenotype (Saka et al., 1994), and Ark1 and Bir1 are indeed required for the correct intranuclear localization of condensin subunits during mitosis (Morishita et al., 2001; Petersen and Hagan, 2003).

In meiosis, Aurora-B kinases have been shown to regulate cohesion in *Caenorhabditis elegans* and *Drosophila melanogaster*. In the worm, the Aurora-B kinase AIR-2 promotes segregation of homologous chromosomes, presumably by phosphorylation-dependent removal of meiotic cohesin, which contains the meiosis-specific subunit Rec8 that replaces Rad21 (Kaitna et al., 2002; Rogers et al., 2002). In *Drosophila*, Aurora-B seems to be required to preserve centromeric cohesion beyond meiosis-I, depending on MEI-S332, a member of the Shugoshin family of proteins (Resnick et al., 2006). The only indication for a role of Aurora in controlling chromosome attachment in meiosis comes from budding yeast, where very recent work has shown that Ipl1 is required for the bi-orientation of homologous chromosomes (Monje-Casas et al., 2007; Yu and Koshland, 2007). Here, we examined the role of the fission yeast Aurora kinase Ark1 in chromosome segregation during mitosis and meiosis. We find that Ark1 is required to promote the bi-orientation of chromosomes in mitosis and to prevent or correct syntelic and merotelic attachment. Furthermore, Ark1 is necessary for the bi-orientation of homologs in meiosis-I. However, notably different from budding yeast (Monje-Casas et al., 2007), we describe that fission yeast Aurora is required for the monopolar attachment of sister chromatids in meiosis-I, and acts in a different pathway from the Moa1/Rec8 mono-orientation pathway that has been defined.

**Results**

**Ark1 ensures faithful centromere segregation in mitosis**

To address whether Ark1 has a role in the bi-orientation of chromosomes, we examined chromosome segregation in haploid fission yeast, in which chromosome 2 was marked with GFP close to the centromere (cen2-GFP; Yamamoto and Hiraoka, 2003) using a temperature-sensitive allele of *ark1* (ark1-T7; Kawashima et al., 2007) or analog-sensitive versions of Ark1 (ark1-as2, ark1-as3, see Materials and methods), which can be inactivated by specific inhibitors (4-amino-1-tert-butyl-3-(1’-napthyl)pyrazolo[3,4-d]pyrimidine (1NA-PP1) or 4-amino-1-tert-butyl-3-(1’-napthylmethyl)pyrazolo[3,4-d]pyrimidine (1NM-PP1)). When Ark1 was inactivated, centromere segregation was perturbed in half or more than half of the cells (Figure 1A). In about 40% of cells, one of the sister chromatids did not move entirely toward one pole in anaphase ('lagging'), and in 11–25% (depending on the allele), both sister centromeres moved to the same pole. This is indicative of merotelic and syntelic chromosome attachment, respectively. To obtain further insight into chromosome movement in *ark1*-mutant cells, we observed chromosome segregation in living *ark1*-T7 cells at the restrictive temperature using cen2-GFP and the spindle pole body marker Sid4-GFP (Figure 1B and C). We found that even before anaphase, alignment of chromosomes on the mitotic spindle was defective, since the cen2-GFP mark was found close to one of the spindle poles in about 60% of the *ark1*-T7 cells at restrictive temperature (Figure 1C, and data not shown). In anaphase, sister chromatids of chromosome 2 missegregated in 28% of the cells. In a few cases (3% of all cells) the sister chromatids stayed entirely at one pole during anaphase. In the other cases, sister chromatids were lagging behind on the spindle during anaphase, with about two-thirds eventually moving to the correct and the remainder moving toward the incorrect pole (Figure 1B and C).

Cells with mutations in condensin fail to segregate the bulk of chromatin very similar to *ark1*-mutant cells, and we therefore wanted to exclude that the chromosome segregation defect observed after Ark1 inhibition is merely a consequence of the condensation defect. When we observed cen2-GFP segregation in temperature-sensitive condensin-mutant cells (*cut3-477*), we also found some failure in centromere segregation (Supplementary Figure S1), which might be attributed to the disturbed structure of the chromosomes (Hirano, 2005). However, this defect in centromere segregation was less pronounced than in *ark1*-mutant cells, although the chromatin condensation defect in these two strains was similar (Supplementary Figure S1). Furthermore, in the presence of low concentrations of the microtubule-destabilizing substance TBZ, *ark1*-T7 cells exhibited considerable missegregation (about 35%) even at the permissive temperature when chromosome condensation was largely normal (Supplementary Figure S1). In contrast, in wild-type or *cut3*-mutant cells, TBZ only caused a very minor increase in missegregation (Supplementary Figure S1). This suggests that Ark1 is needed to establish proper microtubule-kinetochore attachment independent of its role in chromosome condensation. Ark1 also functions in the mitotic spindle checkpoint (Petersen and Hagan, 2003; data not shown). However, even the additional deletion of the checkpoint gene *mad2* in *cut3-477* cells leads to a weaker defect in chromosome segregation than the one observed in *ark1*-T7 cells (Supplementary Figure S1). Taken together, these results indicate that Ark1 plays a specific role in promoting proper chromosome segregation beyond its role in the mitotic checkpoint and in chromosome condensation.

**Ark1 is required for the correction of malattachment**

In budding yeast and metazoans, Aurora kinases act in the bi-orientation of chromosomes by correcting improper, syntelic attachment of chromosomes to the same spindle pole (Tanaka et al., 2002; Haul et al., 2003; Lampson et al., 2004). When we inactivated Ark1 in an otherwise unperturbed mitosis, segregation of both sisters to one spindle pole was rare (Figure 1). This could be because in an unperturbed mitosis, initial syntelic attachment is rare or because syntelic attachment can still be corrected in *ark1*-mutant cells. We therefore increased the frequency of misattachment by first arresting cells in mitosis without microtubules, using a cold-sensitive tubulin mutant *nda3-KM311* and then releasing to permissive temperature (Trautmann et al., 2004; Grishchuk and McIntosh, 2006; Kawashima et al., 2007). Because *ark1*-mutant cells do not arrest in mitosis under these conditions (Petersen and Hagan, 2003; data not shown), we could only perform the experiment with the *ark1*-as3 allele, which is functional when cells are grown without inhibitor and thus allows arrest by *nda3-KM311*. Shortly before release, we
inhibited Ark1-as3 by the specific inhibitor 1NM-PP1, which inactivates the kinase within 10 min (data not shown and Figure 2A). When checking anaphase spindles 10 min after the release, both cen2-GFP marks were found close to one edge of the spindle in about 20% of cells. No such missegregation was observed in wild-type cells treated with inhibitor or in ark1-as3 cells grown without inhibitor (Figure 2A), indicating that its occurrence depends on the inactivation of Ark1. To determine whether this indeed represents initial misattachment that fails to correct, we filmed Ark1-inactivated cells being released from the ndz3 arrest. Among those cells that we could image from the start of mitosis, about half had both sister centromeres located entirely at one pole throughout prometaphase and anaphase (Figure 2B and C; Supplementary Figure S2). These data indicate that Ark1 is required to correct syntelic misattachment of chromosomes (also see Supplementary Note 1).

To confirm in a different setting that malattachment of chromosomes cannot be corrected in ark1-mutant cells, we used a temperature-sensitive mutant of fission yeast cohesin, psc3-1T (Nonaka et al, 2002). Sister chromatids precociously detach from each other in the absence of cohesin, but remain competent to attach to microtubules. However, in the absence of cohesion between sister chromatids, kinetochore-microtubule attachment cannot be stabilized because of a lack of tension. Therefore, attachment remains unstable, which in budding yeast depends on Ipl1 (Biggins and Murray, 2001). In live-cell microscopy experiments, the instability of sister chromatid attachment in psc3-1T cells was exemplified by an occasional switching of at least one sister chromatid from one spindle pole to the other (Figure 2D, arrows). When Ark1 was additionally inactivated, such switching was abolished (Figure 2D), indicating that Ark1 is required to keep sister chromatid attachment unstable in psc3-1T cells. This result is consistent with Ark1 being needed to correct kinetochore-microtubule attachments that fail to generate tension through bi-orientation. However, the fact that sister chromatids were not very motile when Ark1 is inhibited (Figure 2D) could also indicate a more profound defect in kinetochore-microtubule dynamics. Inactivation of condensin did not seem to have the same effect as Ark1 inactivation (Supplementary Figure S2B), which again suggests that Ark1 regulates chromosome attachment independent of condensin.

**Ark1 has a role in nuclear division during meiosis-I**

To study the requirement of Ark1 in meiosis, we used an ‘ark1 shut-off’ (ark1 s.o.) strain, where the ark1+ gene is inhibited by the specific inhibitor 1NM-PP1, which inactivates the kinase within 10 min (data not shown and Figure 2A). When checking anaphase spindles 10 min after the release, both cen2-GFP marks were found close to one edge of the spindle in about 20% of cells. No such missegregation was observed in wild-type cells treated with inhibitor or in ark1-as3 cells grown without inhibitor (Figure 2A), indicating that its occurrence depends on the inactivation of Ark1. To determine whether this indeed represents initial misattachment that fails to correct, we filmed Ark1-inactivated cells being released from the ndz3 arrest. Among those cells that we could image from the start of mitosis, about half had both sister centromeres located entirely at one pole throughout prometaphase and anaphase (Figure 2B and C; Supplementary Figure S2). These data indicate that Ark1 is required to correct syntelic misattachment of chromosomes (also see Supplementary Note 1).

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under control of the mitosis-specific promoter of the rad21+ gene. In this strain, the level of Ark1 protein in meiosis was largely reduced, but minor amounts of Ark1 were still observed at centromeres and midspindles (Supplementary Figure S4). For some experiments, we therefore either additionally or alternatively used the ark1-as2 allele together with the specific inhibitor.

The most prominent phenotype observed after Ark1 depletion in mitosis is a failure to condense chromosomes, which leads to a defect in nuclear division (Petersen et al., 2001). We similarly found a defect in chromosome condensation during meiosis in ark1 s.o. cells (Supplementary Figure S5). Nevertheless, four distinct, albeit often unequally sized, nuclei formed after meiosis-II in ark1 s.o. cells (Supplementary Figure S5). However, in about 50% of ark1 s.o. cells, the two meiosis-II spindles formed extremely close to each other and often in what seemed to be one nucleus (Figure 3A), indicating a failure of nuclear division during meiosis-I. To assess this phenotype better, we arrested cells after meiosis-I using the mes1 mutation (Izawa et al., 2005). Under these conditions, more than 80% of ark1+ cells but only 30% of ark1 s.o. cells formed two nuclei (Figure 3B and C). Nevertheless, the mononucleated ark1 s.o. cells seemed to have undergone anaphase-I, because Rec8-GFP was largely removed from chromatin (Supplementary Figure S6). The failure in nuclear segregation after Ark1 depletion

![Figure 2](image-url)

**Figure 2** Ark1 is required for the correction of malattachment. (A) The indicated strains carrying the nda3-KM311 mutation were arrested in mitosis by incubation at 19°C for 6 h, and released by transfer to 32°C. Where indicated (+ inhib.) 5 μM 1NM-PP1 were added to the culture 10 min before release. Cells were fixed with methanol 10 min after upshift to 32°C. Chromosome segregation was assessed by determining cen2-GFP localization on anaphase spindles, which were labeled by mCherry-Atb2 (tubulin). (B, C) nda3-KM311 ark1-as3 cells were arrested in mitosis as in panel A and released by transferring cells to a microscope stage kept at 32°C. 1NM-PP1 (5 μM) was added 5 min before release. Only cells that could be followed through mitosis starting from very short spindle length were considered. Cells in which the spindle was defective and those in which centromere 2 did not attach to the spindle were excluded from the analysis. A kymograph of a cell that showed chromosome 2 segregating with one SPB is shown in panel C. Example kymographs for all phenotypes as well as example kymographs from ark1-as3 cells released from the nda3-KM311 arrest without any inhibitor are shown in Supplementary Figure S2A. (D) The indicated strains marked with cen2-GFP and mCherry-Atb2 (tubulin) were followed by live-cell microscopy at the restrictive temperature (34°C). A centromere was considered to have ‘switched’ when the trajectory of cen2-GFP from one pole to the other could be clearly observed (arrows). The number of ‘switches’ per hour in a total of 2 h prometaphase observation time is given.
presumably reflects a failure in condensin function, since mutants in the condensin subunit *cut3* also show a slightly increased number of cells with one nucleus when arrested by the *mes1* mutation at a semi-permissive temperature (data not shown).

Ark1 promotes homolog bi-orientation in meiosis-I

To address whether Ark1 has any role in meiotic chromosome segregation, we examined the segregation of *cen2*-GFP in *ark1* s.o. and *ark1-as2* cells during anaphase-I. When Ark1 was inhibited or depleted, both homologous chromosomes 2 segregated to the same pole in about 30–40% of anaphase-I cells (Figure 4A). In another 30% of cells, at least one of the homologs was lagging on the anaphase-I spindle. These segregation problems could be caused either by a failure to bi-orient bivalents or by a failure to join homologous chromosomes through chiasmata. Since the intergenic recombination frequency between the *lys3*+ and *ura1*+ locus on chromosome 1 was similar in *ark1*+ and *ark1* s.o. strains (data not shown), the latter is unlikely. Therefore, these data suggest that Ark1 is required for the bi-orientation of connected homologs during meiosis-I (also see Supplementary Note 2).

The role of Ark1 in bi-orientation is independent of kinetochore geometry

Paliulis and Nicklas (2000) have shown that specific features of the chromosome and not of the spindle determine the special chromosome segregation of meiosis-I. In fission yeast,
genetic tricks can be used to create mitosis-like chromosomes during meiosis-I. Deletion of the gene for the meiosis-specific cohesin subunit Rec8 causes a failure in recombination and therefore chiasmata generation. Additionally, sister kinetochores in each homolog are faithfully bi-oriented presumably because geometry at the centromeric region is mitosis-like and sister chromatids remain cohered by mitotic cohesin complexes, which persist into meiosis (Yokobayashi et al., 2003). Similarly, mitosis-like chromosomes can be generated by deleting rec12+, which is required for recombination, and moa1+, the gene product of which is required for the mono-orientation of sister kinetochores in concert with Rec8 (Yokobayashi and Watanabe, 2005). In both these genetic backgrounds, Ark1 shut-off caused mis-segregation of sister centromeres in meiosis-I (Figure 4B), with the effect being greater in a rec12 Δ moa1Δ strain than in a rec8Δ strain (see Supplementary Note 3). Thus, during meiosis-I, Ark1 can promote the equational segregation of sister chromatids (Figure 4B), or the bipolar segregation of homologs (Figure 4A), depending on the chromosome structure. We suggest that Ark1 promotes the bi-orientation of any two kinetochore-containing entities that are connected. This is in accordance with findings by Dewar et al. (2004), who showed that budding yeast Ipl1 ensures the bi-orientation of two separate kinetochores on an unreplicated plasmid.

Ark1 is required for the mono-orientation of sister chromatids in meiosis-I

When we tested the segregation of homologs during anaphase-I, we found that frequently at least one of the homologs was lagging and often the cen2-GFP signal of lagging homologs split in two (Figure 4A). We therefore hypothesized that the sister kinetochores on one homolog, which normally attach to only one spindle pole, were pulled in opposite direction. To verify this assumption, we labeled only one of the homologous chromosomes 2 with GFP and determined the segregation pattern. Indeed, in about 12% of anaphase-I cells, the GFP signal split in two when Ark1 was depleted (Figure 5A), indicating that sister chromatids were pulled to opposite poles and separated precociously. These data suggested that in the absence of Ark1, sister kinetochores erroneously became attached to opposite spindle poles during meiosis-I. In accordance, we found that a visible separation of sister centromeres could already be observed during metaphase-I (Supplementary Figure S7). We therefore considered the possibility that Ark1 is required for the localization and function of Moa1, which prevents bi-orientation of sister chromatids at meiosis-I (Yokobayashi and Watanabe, 2005). However, visualization of Moa1-GFP did not reveal any difference between wild-type and ark1 s.o. cells during meiosis-I (Figure 5B). Similarly, no significant difference in Moa1 localization was observed by chromatin

Figure 5 Ark1 is required for proper sister chromatid mono-orientation in meiosis-I independent of Moa1. (A) One of the homologous chromosomes 2 was marked by GFP in the indicated strains and the segregation pattern was determined during anaphase-I using CFP- or mCherry-Atb2 (tubulin) as a marker. (B) Cells from the indicated strains expressing Moa1-GFP and CFP-Atb2 (tubulin) were observed during metaphase-I. (C) Diploid strains of the indicated genotype were arrested in prophase-I by deletion of mei4+, and the amount of Moa1 at centromeres was determined by ChIP using a Moa1 antibody.
immunoprecipitation (ChIP; Figure 5C). Furthermore, the localization of Rec8 at the centromeric central core, which is important for mono-orientation of kinetochores (Watanabe et al, 2001; Yokobayashi et al, 2003), was intact in the arkl s.o. cells (Supplementary Figure S8). Thus, the mono-orientation defect caused by the reduction of Ark1 is likely different from the one caused by the absence of Moa1 or Rec8, suggesting that Ark1 and Moa1/Rec8 influence sister kinetochore mono-orientation in meiosis-I through distinct mechanisms.

**Sister centromeres separate precociously after Ark1 depletion because of merotelic attachment rather than complete bi-orientation**

Cells in which moa1+ is deleted show entirely equational segregation of sister chromatids in meiosis-I if recombination is abolished by rec12A (see Figure 4B). In this situation lagging chromatids can be observed in anaphase-I. Their appearance is completely suppressed by deleting sgo1+, the meiosis-I-specific protector of centromeric cohesion (Yokobayashi and Watanabe, 2005). In contrast, neither deletion of rec12+ nor the additional deletion of sgo1+ leads to completely equational segregation in arkl s.o. cells (Supplementary Figure S8C, and data not shown). When contemplating the reason for the mono-orientation defect in arkl s.o. cells, we noticed the high frequency of lagging chromatids or lagging sister centromidats during anaphase-I (Figures 4A and 5A). Given that lagging chromatids may originate from merotelic attachment and Ark1 is involved in its correction (Figure 1), we envisaged that the primary defect in arkl s.o. cells preventing monopolar attachment could be the inability to correct merotelic attachment of a unified pair of sister kinetochores (Figure 6B). In rec12A moa1Δ cells, all sister centromeres eventually segregated at anaphase-I even in the presence of Sgo1, implying that microtubule-mediated pulling on bi-orientated sister centromeres can overcome Sgo1-mediated protection (Vaur et al, 2005; Yokobayashi and Watanabe, 2005; Figure 6B). The tension on homologs that are attached in a merotelic manner is expected to be less, because some microtubules on both sister kinetochores likely attach to the same pole (Figure 6B, ‘sgo1+’). This reduced tension might not be sufficient to overcome protection by Sgo1. This hypothesis makes the key prediction that deprotection of sister chromatid cohesion by sgo1Δ would increase the equational segregation of sister centromeres in arkl s.o. cells, different from rec12Δ moa1Δ cells (see Figure 6B). Consistent with this scenario, the ratio of cells in which sister centromeres were moving entirely to opposite poles in anaphase-I increased from ~1% in arkl s.o. to 17% in arkl s.o. sgo1Δ cells (Figure 5A). Furthermore, lagging sister chromatids could be observed in arkl s.o. cells even after sgo1+ deletion, indicating that single chromatids were attached in a merotelic manner (Figures 5A and 6B, ‘sgo1Δ’).

Thus, we suggest that Ark1 promotes monopolar attachment of sister kinetochores at meiosis-I most likely by correcting merotelic attachment of paired sister kinetochores, a mechanism that is fundamentally different from that of Moa1 and Rec8, which are thought to promote the side-by-side orientation of sister kinetochores by fostering cohesion in the central core region of the centromere (Figure 6B).

![Figure 6](https://example.com/figure6.png)

**The Bub1–Sgo2–Ark1 pathway operates for proper chromosome segregation in meiosis**

Recent reports indicated that the Shugoshin protein Sgo2 interacts with the CPC protein Bir1 and is required for the full recruitment of the CPC including Ark1 to centromeres (Kawashima et al, 2007; Vanooosthuyse et al, 2007). Cells depleted of Sgo2 exhibit non-disjunction of homologs in about 20% and equational segregation of sister centromeres in ~5% at meiosis-I (Kitajima et al, 2004; Rabitsch et al, 2007).
Since localization of Ark1 at centromeres is reduced in sgo2Δ cells at meiosis-I ( Supplementary Figure S9; Kawashima et al., 2007 ), the non-disjunction of homologs in sgo2Δ cells was attributed to defects in Ark1 function. Based on the results described above, we hypothesized that the mono-orientation defect in sgo2Δ cells originates from the inability to correct merotelic attachment of paired sister kinetochores, like in ark1 s.o. cells. Supporting this assumption, an attempted separation of sister centromeres at anaphase-I was observed in about 16% of sgo2Δ cells, which is very similar to that in ark1 s.o. cells ( Figure 5A ). Moreover, deletion of sgo1 + increased the equational segregation of sister centromeres of chromosome 2 at meiosis-I to 12% ( Figure 5A; also see Figure 6A ), which is again very similar to that in ark1 s.o. cells. Thus, we conclude that defects in meiotic chromosome segregation in sgo2Δ cells are mostly caused by reduced Ark1 function. In contrast to the chromosome segregation function, the role of Ark1 in promoting chromosome condensation is not shared by Sgo2 ( Supplementary Figure S5 ).

Previous data supported the view that Bub1 acts upstream of Sgo1 and Sgo2 (Kitajima et al., 2004; Supplementary Figure S9). Fittingly, Ark1-GFP localization was perturbed during metaphase-I to a similar extent as in sgo2Δ cells by deletion of bub1 + ( Supplementary Figure S9E and F ). Furthermore, we found that deletion of sgo2 + did not enhance the mono-orientation defect of bub1Δ cells ( Figure 6A ), indicating that Bub1 and Sgo2 work in a single pathway. These results suggest that the defects in mono-orientation after Bub1 depletion might be partly caused by the inability to correct merotelic attachment of paired sister kinetochores, similar to the situation in ark1 s.o. or sgo2Δ cells (see Discussion).

Discussion

Mitotic functions of Aurora are conserved in fission yeast

Aurora kinases are highly conserved throughout eukaryotes, and have been implicated in proper chromosome segregation in several organisms (Vagnarelli and Earnshaw, 2004). Noticeably, however, in fission yeast, a well-studied model in mitosis research, it was unknown whether the single Aurora kinase, Ark1, has any role in regulating the proper attachment of chromosomes during mitosis. Here, we demonstrate that Ark1 inhibition causes misattachment of chromosomes to the mitotic spindle (Figure 1). As in budding yeast (Tanaka et al., 2002), Aurora seems necessary to destabilize syntelic attachment that fails to create tension ( Figure 2 ). In addition, lagging chromatids occurred with high frequency when Ark1 was inhibited ( Figure 1 ). Those might arise because attachment to microtubules is weak or dysfunctional, or because the corresponding kinetochore is attached to both spindle poles (merotelic attachment). We favor the latter hypothesis, since most sister chromatids in ark1-T7 cells move to the spindle poles in anaphase-A with velocities comparable to those observed in wild-type cells (data not shown), indicating that there is no general problem with attachment or microtubule-dependent anaphase movement. In vertebrate cells, where kinetochore–microtubule attachment can be visualized directly, it has been shown that Aurora-B is required to suppress merotelic attachment, possibly by destabilizing the faulty attachment (Cimini et al., 2006; Knowlton et al., 2006). We consider it an additional possibility that Aurora is required to build the kinetochore in a way that favors attachment of all microtubules on one kinetochore to the same pole. It has been proposed that fission yeast Pcs1, which is a homolog of one of the components of the budding yeast monopolin complex, is required to clamp together single microtubule-binding sites on one kinetochore, thus favoring their attachment to one pole (Rabitsch et al., 2003). Indeed, deletion of pcs1 + causes kinetochores to attach in a merotelic manner (Rabitsch et al., 2003; Gregan et al., 2007). Thus, Ark1 might be required for Pcs1 function. Our preliminary experiments nevertheless failed to detect an influence of Ark1 on Pcs1 localization (data not shown).

In addition to its functions in regulating kinetochore attachment, budding yeast Ipl1 is a component of the ‘NoCut’ pathway, which prevents abscission in the presence of spindle-midzone defects (Norden et al., 2006). In our experiments it was evident that an equatorial microtubule ring, which normally forms during mid or late anaphase-B in the plane of cell division (Pichova et al., 1995; Heitz et al., 2001), was formed precociously when Ark1 was inhibited ( Supplementary Figure S2A ), thus also implying Ark1 in the regulation of cytokinesis.

Ark1 acts on chromosome attachment in a similar way during mitosis and meiosis

Since Ark1 promotes sister chromatid bi-orientation in mitosis, but sister chromatids have to mono-orient during meiosis-I, it was unclear how Ark1 would influence chromosome segregation in meiosis-I. We found that Ark1 normally promotes the bi-orientation of homologs in meiosis-I, but if the morphology of the bivalent is disrupted and mitosis-like chromosomes are created, Ark1 promotes the bi-orientation of sister chromatids ( Figure 4 ). This indicates that the molecular mechanism of Ark1 function is the same in mitosis and meiosis, and the different outcome is determined by the structure of the bivalent.

In contrast to mitosis, where Ark1 inhibition causes only about 10–20% sister chromatid co-segregation, depletion of Ark1 in meiosis causes a more pronounced co-segregation of homologous chromosomes (30–40%). The most likely explanation is that tension-controlled correction of attachment is more important in meiosis, because the two pairs of sister kinetochores on a bivalent are not as tightly coupled as the sister kinetochores on a mitotic chromosome, which might favor their syntelic attachment ( Shonn et al., 2000 ).

Very recently it has been demonstrated that the budding yeast Aurora kinase, Ipl1, is similarly required for homolog bi-orientation and sister chromatid bi-orientation of artificial mitosis-like chromosomes in meiosis-I (Monje-Casas et al., 2007). Because of the high conservation of Aurora functions in all eukaryotes, we expect that this will also hold true for metazoans.

Does Ark1 facilitate cleavage of Rec8?

In C. elegans, the Aurora-B kinase AIR-2 seems to be required to efficiently remove cohesin complexes containing Rec8 from chromosome arms (Kaitna et al., 2002; Rogers et al., 2002). In contrast, we find that Ark1 is not essential for Rec8 removal during meiosis-I in fission yeast ( Supplementary Figure S10 ). In time-lapse movies of fission yeast expressing...
Rec8-GFP, one can clearly observe the solubilization of Rec8 at the onset of anaphase-I, presumably at the moment when it is cleaved and removed from chromatin (Supplementary Figure S10). Although this step is not as easy to discern when Ark1 is inhibited, because chromosomes are less condensed, it is clear that this solubilization also happens fairly efficiently if Ark1 is inhibited and Rec8 is subsequently degraded with kinetics similar to wild-type cells (Supplementary Figure S10). Nevertheless, it is possible that Ark1 facilitates but is not essential for Rec8 cleavage (see Supplementary Note 3).

A novel mechanism to promote mono-orientation of sister chromatids during meiosis-I

We show that fission yeast Ark1 is required for the faithful mono-orientation of sister chromatids in meiosis-I (Figure 5). In fission yeast, mono-orientation of sister kinetochores is regulated by Moa1 and Rec8, which may cooperatively promote the formation of a side-by-side structure of sister kinetochores through cohesion of the centromeric core region (Yokobayashi and Watanabe, 2005). The depletion of Moa1 together with Rec12 or of Rec8 entirely disrupts the mono-orientation of sister chromatids at meiosis-I. The mono-orientation defect in ark1 s.o. cells is less pronounced, and Moa1- or Rec8-localization is not disrupted when Ark1 is depleted (Figure 5; Supplementary Figure S8). This indicates that Ark1 and Moa1 act in separate pathways to promote mono-orientation. Our experiments suggest that in the absence of Ark1, sister kinetochores on one homolog become attached in a merotelic manner so that they are torn apart at anaphase-I, even though they have the proper side-by-side configuration that favors mono-orientation (Figure 6B). Thus, the complicated chromosome segregation defects in meiotic cells depleted of Ark1 can be explained by the well-recognized role of Aurora in correcting malattachment of chromosomes. Syntelic and merotelic attachment of bivalents in meiosis-I provokes non-disjunction of homologs and precarious sister separation, respectively.

In budding yeast, it has been proposed that the two pairs of sister kinetochores on a bivalent only attach to one microtubule each (Winey et al., 2005) and one sister kinetochore may thus be inactivated. Consequently, merotelic attachment might not be possible, which would explain the unperturbed mono-orientation in Ipl1-depleted cells (Monje-Casas et al., 2007) despite the otherwise similar function of Ipl1 and Ark1. There is, however, controversy in the literature whether the depletion of Ipl1 causes a mono-orientation defect (Yu and Koshland, 2007). In any case, our data clearly indicate that in fission yeast, both sister kinetochores are active in meiosis-I and can attach to microtubules. As attachment of both kinetochores to microtubules at meiosis-I is observed in several organisms (Lee et al., 2000; Parra et al., 2004), the mechanism we identified here may be functional in other eukaryotes as well.

The Bub1–Sgo2–Ark1 pathway ensures meiosis-I chromosome segregation by correcting malattachment of homologs

Our finding that Ark1 is needed for the bi-orientation of homologs and the mono-orientation of sister kinetochores in meiosis-I provides a crucial clue to solve the enigma why Sgo2 and Bub1 are required for monopolar attachment as well as proper homolog disjunction in meiosis-I. In either sgo2Δ or bub1Δ cells, centromeric localization of Ark1 is reduced at meiosis-I (Supplementary Figure S9). This is consistent with previous findings that Bub1 acts upstream of Sgo2, which in turn plays a crucial role to load the CPC to centromeres (Kitajima et al., 2004; Kawashima et al., 2007; Vanoosthuyse et al., 2007). Since Moa1 localization is intact in either sgo2Δ or bub1Δ cells (data not shown), it is reasonable to assume that perturbation of mono-orientation in these cells may originate from the reduced Ark1 activity at centromeres. Indeed, the defects of sgo2Δ cells in monopolar attachment at meiosis-I resemble those of ark1 s.o. cells (Figure 5A). Because bub1Δ cells are defective in both Sgo1 and Sgo2 localization to centromeres (Kitajima et al., 2004; Supplementary Figure S9), one would expect that bub1Δ phenocopies the sgo1 + sgo2 + double deletion. However, the frequency of equational segregation is significantly higher in bub1Δ cells (~30%) than sgo2Δ sgo1Δ cells (12%) (Figure 6A; Bernard et al., 2001). Since in mitosis bub1Δ cells show a higher number of lagging chromosomes than sgo2Δ cells (Bernard et al., 1998; S Kawashima and Y Watanabe, unpublished results), we suggest that Bub1 has functions that go beyond Sgo2 regulation both in mitosis and meiosis. Whatever the nature of these additional functions is, our results argue that Bub1 depletion perturbs monopolar attachment by generating merotelic attachment of paired sister kinetochores, like Sgo2 or Ark1 depletion.

In summary, we have shown that the conserved functions of Aurora for correcting malattachment are acting in fission yeast mitosis and meiosis. Furthermore, we demonstrate that this activity of Aurora is required to ensure monopolar attachment at meiosis-I, and we expect that the same will hold true for metazoans.

Materials and methods

S. pombe strains

All strains used in this study are listed in Supplementary Table 1 in Supplementary data. To generate the ark1-as2 allele (Ark1-::Leu166Ala; Bishop et al., 2000), the ark1 gene was PCR-mutagenized from a strain into which a hygromycin-resistance cassette (hygR) had been integrated 400 bp 5′ of the ark1 + open reading frame (ORF). The hygR-ark1-as2 construct was integrated in a wild-type strain at the endogenous locus. The ark1-as2 allele rendered the cells sensitive to 5 μM 1NA-PP1 or 5 μM 1NM-PP1 (both from TRC, North York, ON, Canada). The ark1-as3 strain was created from ark1-as2 by additionally mutating Ser229 to Ala. Both the ark1-as2 and the ark1-as3 strain used in this study contain the additional amino-acid mutations Gln28Arg and Gln176Arg, which may cooperatively affect the activity of Aurora controls meiotic chromosome segregation

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The EMBO Journal VOL 26 | NO 21 | 2007 4483
et al., 2002; Yamamoto and Hiraoka, 2003; Grallert et al., 2004; Kitajima et al., 2004; Yokobayashi and Watanabe, 2005; Kawashima et al., 2007).

**Culture conditions**
Medium for mitotic cultures was YE (YE with additional 50 mg/l adenine) or minimal medium (MM) containing 5 g/l NH₄Cl and additional nutrients if required (Alfa et al., 1993). To synchronize cells in mitosis (Figure 1A), we arrested cells in S-phase by incubation in 12 mM hydroxyurea for 4.5 h at 25 or 30°C depending on the strain. The arrest was released by washing the cells twice with fresh, warm medium before reculturing. Cells carrying the ndz3-KM311 mutation (Figure 2A and B) were arrested in mitosis by incubation at 19°C for 6 h and subsequently released by shifting to 32°C.

To observe cells in meiosis, cells were first grown to logarithmic phase. If the nmt41 or nmt81 promoter should be induced by thiamine depletion, cells were grown in MM containing 5 g/l NH₄Cl and, if necessary, 200 mg/l leucine and 50 mg/l adenine for about 14 h at 30°C. Cells were washed, collected and spotted on sporulation agar (SPA; Gutz et al., 1974) to which leucine or adenine had been added if necessary. After a further 7–8 h of incubation at 30°C, cells were observed directly or fixed by methanol at —80°C. To observe arkl-as2 cells in meiosis, the cells were first incubated in MM with 5 g/l NH₄Cl for 8–9 h, and then washed and incubated in MM without NH₄Cl for 4–5 h before spotting on a plate with synthetic sporulation agar (SSA; SSL with agar; Egel, 1971) containing 5 μM 1NA-PP1. Cells were observed after 7–10 h of incubation at 30°C.

**Immunostaining and DNA staining**
For immunostaining, cells were fixed with paraformaldehyde. To stain microtubules, we used the mouse anti-tubulin TAT1 antibody (kind gift from K Gull) at a dilution of 1:200, followed by Alexa568-coupled anti-mouse secondary antibody (Invitrogen) at 2 μg/ml. To stain DNA, methanol-fixed cells were washed, resuspended in PEM buffer (100 mM PIPES, 5 mM EGTA, 5 mM MgCl₂, pH 6.9) and stained by 1 μg/ml Hoechst 33342 or 1 μg/ml DAPI.

**Image acquisition**
Images were acquired on a Zeiss AxioImager microscope (Zeiss, Jena, Germany) with MetaMorph software (Molecular Devices, CA). To observe cells in meiosis, cells were first grown to logarithmic phase. If the nmt41 or nmt81 promoter should be induced by thiamine depletion, cells were grown in MM containing 5 g/l NH₄Cl and, if necessary, 200 mg/l leucine and 50 mg/l adenine for about 14 h at 30°C. Cells were washed, collected and spotted on sporulation agar (SPA; Gutz et al., 1974) to which leucine or adenine had been added if necessary. After a further 7–8 h of incubation at 30°C, cells were observed directly or fixed by methanol at —80°C. To observe arkl-as2 cells in meiosis, the cells were first incubated in MM with 5 g/l NH₄Cl for 8–9 h, and then washed and incubated in MM without NH₄Cl for 4–5 h before spotting on a plate with synthetic sporulation agar (SSA; SSL with agar; Egel, 1971) containing 5 μM 1NA-PP1. Cells were observed after 7–10 h of incubation at 30°C.

**Time-lapse imaging**
Live-cell recordings were performed on a DeltaVision RT system (Applied Precision, Issaquah, WA) equipped with a heating chamber. For imaging mitosis in arkl1-mutants, cells were grown in liquid medium at permissive temperature, transferred to a glass-bottom culture dish (MatTek, Ashland, MA) coated with lectin and incubated on the microscope stage at the restrictive temperature (34°C) for at least 1 h, before starting image acquisition. To image cells in an ndz3-KM311 release, cells were transferred from a liquid culture at 19°C to a glass-bottom culture dish coated with lectin, which was placed in the microscope chamber heated to 32°C. Image acquisition was started immediately. Images were acquired with the Z-sweep acquisition (OAI) feature and deconvolved using softWoRx software. Kymographs were assembled with Adobe Photoshop and Image Ready software.

**Chromatin immunoprecipitation**
The procedure was carried out essentially as described previously (Yokobayashi et al., 2003; Kawashima et al., 2007). Anti-Moa1 polyclonal antibodies, anti-GFP polyclonal antibodies (Living Colors; full-length A.v. Polyclonal Antibody, Clontech) and anti- Cp1 polyclonal antibodies were used for IP (Yokobayashi and Watanabe, 2005). DNA prepared from whole-cell extracts or immunoprecipitated fractions was analyzed by quantitative PCR with ABI PRISM7000 (Applied Biosystems) using SYBR Premix Ex Taq (Perfect Real Time; Takara). The primers used for PCR were described previously (Yokobayashi et al., 2003; Kawashima et al., 2007).

**Supplementary data**
Supplementary data are available at The EMBO Journal Online (http://www.embojournal.org).

**Acknowledgements**
We thank Iain Hagan, Jonathan Millar, Keith Gull and Roger Tsien for sharing strains, antibodies and plasmids; Eva Ilgen for excellent technical assistance; Andri Koch for generating the arkl1-as1 strain; Alwin Kphler, Stephanie Küng and Hanna Windecker for comments on the manuscript; Dmitri Ivanov for discussions and the Watanabe laboratory, in particular Shihori Yokobayashi, Tomoya Kitajima and Takeshi Sakamoto, for support. We are grateful for the financial support by the German Research Foundation (DFG), the Human Frontier Science Program (HFSP) and the Max Planck Society.

**Author contributions**
YW and SH conceived the project; SH and ML designed and performed the mitosis experiments; AB and SH designed and performed most meiosis experiments; SAK contributed the data in Figure 6A; Supplementary Figure S9A, B, E and F; TT performed the ChIP experiments and SH and YW wrote the paper.

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