DNA anaphase bridges are a potential source of genome instability that may lead to chromosome breakage or nondisjunction during mitosis. Two classes of anaphase bridges can be distinguished: DAPI-positive chromatin bridges and DAPI-negative ultrafine DNA bridges (UFBs). Here, we establish budding yeast *Saccharomyces cerevisiae* and the avian DT40 cell line as model systems for studying DNA anaphase bridges and show that TopBP1/Dpb11 plays an evolutionarily conserved role in their metabolism. Together with the single-stranded DNA binding protein RPA, TopBP1/Dpb11 binds to UFBs, and depletion of TopBP1/Dpb11 led to an accumulation of chromatin bridges. Importantly, the NoCut checkpoint that delays progression from anaphase to abscission in yeast was activated by both UFBs and chromatin bridges independently of Dpb11, and disruption of the NoCut checkpoint in Dpb11-depleted cells led to genome instability. In conclusion, we propose that TopBP1/Dpb11 prevents accumulation of anaphase bridges via stimulation of the Mec1/ATR kinase and suppression of homologous recombination.

**Introduction**

Faithful segregation of the genetic material during cell division is crucial for maintenance of genome integrity. The two complements of the genome must be disentangled before migration into the daughter cells in mitosis. This is a topologically challenging process because sister chromatids are frequently catenated or connected by hemicatenanes at the G2–M transition (Lucas and Hyrien, 2000; Lopes et al., 2003; Wellinger et al., 2003; Liberi et al., 2005; Johnson et al., 2009). As a consequence, the separating sister chromatids are often connected by DNA bridges in anaphase. A subset of these DNA anaphase bridges has been linked to chromosomal fragile sites in human cells (Chan et al., 2009; Lukas et al., 2011). Fragile sites are prone to chromosome breakage, deletion, and translocation, and are often associated with cancer and other genetic diseases (Durkin and Glover, 2007; Gandhi et al., 2010).

DNA anaphase bridges can be divided into two classes (Kaulich et al., 2012): chromatin bridges that can be visualized by DAPI staining, and ultrafine DNA bridges (UFBs; Chan et al., 2007), which are refractory to DAPI staining. In mammalian cells, UFBs are bound by the PICH, BLM, and FANCM helicases, and a subset of UFBs are marked by the Fanconi anemia (FA) proteins, FANCD2 and FANCI, which localize to the termini of these UFBs (Chan et al., 2009; Naim and Rosselli, 2009; Vinciguerra et al., 2010). A subset of BLM-stained UFBs also contain replication protein A (RPA), indicating that some bridges are at least partially single stranded (Chan and Hickson, 2009). In contrast to UFBs, chromatin bridges contain nucleosomes and other chromatin components.

Several models have been suggested to explain the origin of UFBs (Chan and Hickson, 2011). The FA-negative UFBs are the most abundant in unperturbed cells. They originate primarily from centromeric regions and are induced by topoisomerase II inhibition, suggesting that they reflect catenated sister chromatids. The FA-positive UFBs are rare in unperturbed cells, are induced by inhibition of DNA replication, and originate...
primarily at common fragile sites (Chan et al., 2009; Naim and Rosselli, 2009). Although BLM is known to process DNA recombination structures, UFBs are unlikely to reflect recombination intermediates such as Holliday junctions because FANC-D2 foci and UFB formation are independent of the RAD51 recombinase (Chan et al., 2009; Lahkim Bennani-Belhaj et al., 2010).

Chromosomal fragile sites are often marked by 53BP1 in G1 when cells have been exposed to mild replication stress in the previous S phase, indicating that these sites may represent single-stranded gaps originating from incomplete DNA replication (Lukas et al., 2011). The latter study suggested that no checkpoint exists to detect and prevent onset of mitosis in the presence of unreplicated regions of the genome. However, in yeast, lagging chromatin across the spindle midzone, which could be a consequence of unreplicated DNA, was shown to activate a NoCut checkpoint that delays abscission until the sister chromatids are fully segregated (Mendoza et al., 2009). The NoCut checkpoint requires the Ipl1/Aurora B kinase, the spindle-associated factor Slk19, and the Ahc1 histone acetyltransferase (Mendoza et al., 2009). Similarly, in human cells Aurora B was shown to delay abscission in cells with chromosome bridges (Steigemann et al., 2009).

In this study, we report that the DNA damage checkpoint, replication and repair protein Dpb11 localizes to UFBs in budding yeast along with Sgs1-Top3 (BLM-TopoIII), RPA, and the checkpoint protein Ddc2 (ATRIP). We also show that the vertebrate orthologue of Dpb11, TopBP1, colocalizes with PICH and RPA at a subset of UFBs in chicken DT40 cells. Depletion of Dpb11 or TopBP1 leads to an accumulation of chromatin bridges but a reduction in the frequency of long UFBs. UFBs in yeast are sensed by the NoCut checkpoint to delay cytokinesis, and simultaneous disruption of the NoCut checkpoint and depletion of Dpb11 leads to a synergistic increase in genome instability.

**Results**

**Dpb11 localizes to ultrafine anaphase bridges in mitotic cells**

We have recently reported the localization of Dpb11 to DNA double-strand breaks (Germann et al., 2011). In the course of this work, we noticed that Dpb11 localizes to a structure bridging the daughter and mother nuclei in 1–3% of cells in an asynchronous population (Fig. 1 A). When Dpb11 localization was monitored by time-lapse microscopy using 5-min intervals, spontaneous Dpb11 bridges were observed in 43% of anaphases (n = 37). Because this experiment was performed with a tetO::DPB11-YFP construct that overexpresses Dpb11 approximately fourfold compared with the native promoter (Germann et al., 2011), we confirmed that Dpb11 also forms anaphase bridges when expressed from its native promoter (Fig. 1 B). The majority (70–80%) of these bridges fail to stain with conventional DNA dyes (DAPI and Hoechst; Fig. 1, B, C, and F). Further experiments showed that Dpb11 bridges frequently (>80%) colocalize with Rfa1 (RPA; Fig. 1 C), suggesting that these structures at least partially consist of single-stranded DNA, similar to what has been reported for ultrafine DNA bridges in mammalian cells (Chan and Hickson, 2009). To further extend the comparison to mammalian UFBs, we tested the localization of Sgs1-Top3 to Dpb11 bridges. Live-cell imaging showed that Sgs1 and Top3 colocalize with Dpb11 bridges in a subset (60%) of mitotic cells (Fig. 1 D). In contrast, a range of chromatin-associated factors such as Hta1 (histone H2A), Htz1 (histone variant H2AZ), Rsc1 (RSC chromatin remodeling complex), Nhp10 (INO80 chromatin remodeling complex), and Nop1 (nucleolar protein) were generally absent from Dpb11-coated anaphase bridges, indicating that these structures are largely free of chromatin and do not represent the normally late-segregating nucleolus (Fig. 1, E and F; Torres-Rosell et al., 2004).

**Yeast anaphase tubes contain DNA bridges bound by Dpb11**

In contrast to vertebrate cells, the yeast nuclear envelope remains intact during mitosis, causing the nuclear membrane to be stretched into a narrow tube during anaphase as visualized by the nuclear pore complex subunit Nup49 (Fig. 2 A). Therefore, any bridge-like localization of a nuclear protein at this phase of the cell cycle could simply reflect the nucleoplasm contained within the anaphase tube. Indeed, red fluorescent protein tagged with a nuclear localization signal (NLS-RFP) exhibits a bridge-like localization coinciding with Dpb11 bridges (Fig. 2 A). To determine which of the proteins within anaphase bridges are bound to DNA, we performed time-lapse microscopy of dividing Dpb11-YFP cells expressing NLS-RFP and Spc110-CFP to mark the nucleoplasm and spindle pole body (SPB), respectively (Fig. 2 B). Resolution of the Dpb11 bridge before the NLS-RFP marker was observed in 43% of anaphases examined (n = 37), indicating that upon relaxation of the mitotic spindle, the Dpb11 bridge is resolved, whereas the NLS-RFP marker remains in the anaphase tube until nuclear division. These data imply that Dpb11 is associated with DNA at the anaphase bridge. To directly demonstrate the presence of DNA in the anaphase bridges, we constructed a strain that allows incorporation of the nucleoside analogue 5-ethynyl-2′-deoxyuridine (EdU; Viggiani and Aparicio, 2006). This thymidine analogue can be conjugated to a fluorescently labeled azide. Using this technique, we detected the DNA in Hoechst-negative anaphase bridges (Fig. 2 C). Subsequent immunostaining for Dpb11-YFP was incompatible with preservation of the UFBs, but similar to Dpb11-bound UFBs (see following paragraph), the EdU-labeled bridges were induced by mild replication stress (20 mM hydroxyurea), indicating that Hoechst-negative Dpb11 UFBs and EdU-labeled bridges reflect the same structure (Fig. 2 D). Two additional observations were consistent with this conclusion. First, Rfa1 was also bound to UFBs (Fig. S1). Second, disappearance of Dpb11 from bridges coincided with relief of spindle tension, not nuclear division, as seen by the change of SPB movement from poleward to random (Fig. 2, B and E). Furthermore, in cells without a Dpb11 bridge, nuclear division takes place when the SPBs are separated by 4–6 µm, whereas cells containing a Dpb11 bridge exhibit SPB distances of up to 9 µm with a peak at 7 µm (Fig. 2 F), indicating that nuclear division is delayed in cells containing Dpb11 anaphase bridges.

**Dpb11 bridges are induced by DNA replication and topological stress**

To determine the effect of DNA replication stress on the formation of Dpb11 anaphase bridges, we exposed cells to 0.03%...
methyl methanesulfonate (MMS), which blocks replication fork progression through DNA methylation (Tercero and Diffley, 2001). Exposure to MMS led to a transient accumulation of Dpb11 bridges (Fig. 3 A), supporting the notion that DNA replication stress leads to formation of DNA anaphase bridges also in yeast. To monitor the formation of Dpb11 bridges during unchallenged DNA replication, we performed an arrest-release experiment, where Dpb11-YFP localization was monitored after release from α-factor–mediated G1 arrest (Fig. 3 B). In this experiment, Dpb11 bridges appeared at 60 min after release, accumulated until late anaphase at 90 min, and finally disappeared at 120 min, when most of the cells had completed nuclear division (Fig. 3 C). This result indicates that Dpb11 anaphase bridges form in early anaphase and persist until late mitosis. This conclusion was further supported by time-lapse microscopy, which demonstrated that Dpb11 bridges progressively elongate through anaphase. Cells with long anaphase bridges recovered and progressed into the next cell cycle as evidenced by rebudding of both the mother and daughter cell with the same frequency as cells with no bridges or short bridges (Fig. S2).

Top2 catalyzes the decatenation of duplex DNA. To test if catenated chromatids can also lead to anaphase bridges in yeast, we measured Dpb11 bridges in a conditional top2-1 mutant (Brill et al., 1987). Even at the permissive temperature of 25°C, we observed a dramatic increase in the percentage of cells with Dpb11 bridges (Fig. 3 D), indicating that Top2 activity plays a major role in removing anaphase bridges. Although the top2-1 mutant exhibited a basal level of Hoechst-positive chromatin bridges comparable to wild type at the permissive temperature, these were strongly induced at the restrictive temperature (Fig. 3 D). Notably, MMS-induced replication stress and mutation of Top2 additively induced formation of Dpb11-bound anaphase bridges, indicating that topological and replication stress independently contribute to the formation of UFBs (Fig. 3 D). Consistent with a role for Top2 in decatenating intertwined sister chromatids, Top2 itself localized predominantly to Hoechst-negative anaphase bridges (Fig. 3 E). The higher frequency of Top2 bridges (13%) observed in comparison to Dpb11 bridges (4%) could indicate that the function of the Top2-CFP fusion is partially compromised and/or that Top2...
determined the percentage of cells with Dpb11 bridges in an sgs1 mutant. In sgs1 cells the number of chromatin bridges increased, while wild-type levels of UFBs were observed (Fig. 4 A). Taken together, these results show that both DNA replication problems and topological stress are sources of DNA anaphase bridges in yeast.

Homologous recombination promotes the formation of chromatin bridges Another potential source for interlinked sister chromatids is homologous recombination (HR) intermediates. Using mammalian cells it was found that UFB formation is independent of or even increased in the absence of functional RAD51 recombinase (Chan et al., 2009; Lahkim Bennani-Belhaj et al., 2010; Laulier et al., 2011). Similarly, we found that both spontaneous and MMS-induced Hoechst-negative Dpb11 bridges in yeast were largely independent of the Rad52, Rad51, and Rad54...
recombination proteins (Fig. 4, A and B), which is also consistent with our observation that Rad52 binds only to chromatinized Dpb11 bridges (see following paragraph). Interestingly, the chromatin bridges observed in the sgs1 mutant were dependent on HR, indicating that hemicatenated DNA might induce illegitimate recombination leading to formation of chromatin bridges or that Sgs1 suppresses chromatin bridges by dissolution of double-Holliday junctions. Consistent with a role of HR in the formation of Dpb11-bound chromatin bridges, overexpression of Rad51, but not of the catalytically inactive Rad51-K191A mutant, led to increased numbers of Dpb11-marked chromatin bridges (Fig. 4 C). To examine the impact of Dpb11 on anaphase bridges, we took advantage of the Tet-Off promoter (tetO2) to shut off expression of Dpb11 by addition of doxycycline.
Upon repression of \( DPB11 \) expression below the level of detection by fluorescence microscopy, we observed a dramatic increase in the frequency of chromatin bridges. The observed increase in chromatin bridges could reflect either a direct role of Dpb11 in suppressing or resolving chromatin bridges or a defect in DNA replication caused by the low abundance of Dpb11. The observed increase in chromatin bridges depends on HR (Fig. 4 D), which is consistent with the hyper-recombinant phenotype of some \( dpb11 \) mutants (Germann et al., 2011). To determine the frequency of Hoechst-negative anaphase bridges upon Dpb11 repression, we repeated the experiment in a strain expressing Rfa1-CFP as a marker for anaphase bridges. The analysis of this strain confirms that Hoechst-positive bridges accumulate upon Dpb11 depletion, whereas the frequency of Rfa1-marked Hoechst-negative UFBs remains unchanged (Fig. 4 E).

In conclusion, we find that Dpb11-marked UFBs are induced by DNA replication stress in a predominantly recombination-independent manner, whereas recombination promotes the formation of Hoechst-positive chromatin bridges.

Dpb11 ultrafine anaphase bridges activate the NoCut cell cycle delay

The delay in nuclear division observed in cells with a Dpb11 anaphase bridge suggests that a checkpoint may be operating...
DNA damage checkpoint activation in cells with Dpb11 anaphase bridges by monitoring Sml1 protein levels. In response to DNA damage, Sml1 is subject to Mec1-dependent phosphorylation and subsequent ubiquitylation and degradation by the proteasome (Andreson et al., 2010). Surprisingly, the majority of cells with Rad52- and Hoechst-negative Dpb11 bridges exhibit (Fig. 2 F). We decided to test if Dpb11 is required for this checkpoint and if the delay is signaled through stimulation of Mec1 kinase activity (Mordes et al., 2008; Pfander and Diffley, 2011).

We therefore first examined the recruitment of Ddc2 to Dpb11 bridges as a proxy for the Ddc2–Mec1 complex. Similar to Dpb11, Ddc2 is recruited to UFBs (Fig. 5 A). Next, we assessed DNA damage checkpoint activation in cells with Dpb11 anaphase bridges by monitoring Sml1 protein levels. In response to DNA damage, Sml1 is subject to Mec1-dependent phosphorylation and subsequent ubiquitylation and degradation by the proteasome (Andreson et al., 2010). Surprisingly, the majority of cells with Rad52- and Hoechst-negative Dpb11 bridges exhibit...
Recruitment of Dpb11/TopBP1 to UFBs is evolutionarily conserved

PICH-covered UFBs have so far only been observed in mammalian cells. To address whether UFBs also exist in the avian DT40 cell line, we tagged the PICH gene with mTurquoise2 (TFP; Goedhart et al., 2012) or YFP at the endogenous locus. As a marker for chromatin, cells were stably transfected with mCherry-tagged human H2B (hH2B-mCherry). By live-cell microscopy, we readily detected PICH-covered bridges connecting the separating chromosome masses in anaphase cells (Fig. 6 A). Notably, the PICH-coated bridges in untreated DT40 cells neither stained with Hoechst nor did they colocalize with the hH2B-mCherry signal, suggesting that they are uncondensed bona fide UFBs (Baumann et al., 2007; Chan et al., 2009).

The vertebrate orthologue of Dpb11 is TopBP1, which was identified as a topoisomerase IIβ–binding protein ( Yamane et al., 1997). TopBP1 is required for DNA replication and provides a scaffold for DNA damage checkpoint activation ( Mäkiniemi et al., 2001; Lindsey-Boltz and Sancar, 2011). To test the evolutionary conservation of the role of Dpb11 during anaphase, we examined DT40 cells expressing TopBP1-YFP from the endogenous locus. Time-lapse analysis showed that TopBP1 and PICH indeed colocalize at some chromatin-free UFBs from early anaphase until cytokinesis. However, a subset of PICH UFBs are not or only transiently bound by TopBP1 (Fig. 6 B). As mitosis progresses, PICH is the first to dissociate from the UFBs, forming temporary sister foci at the UFB termini (Fig. 6, B and C; and Video 1). In human cells, PICH-bound UFBs and chromatin bridges can be induced by the polymerase α inhibitor aphidicolin (APH) or the topoisomerase II inhibitor ICRF-193 ( Baumann et al., 2007; Chan et al., 2009). We find that ICRF-193 also induces PICH-covered UFBs as well as chromatin bridges in DT40, whereas APH treatment specifically stimulates the formation of chromatin bridges and colocalizing PICH and TopBP1 bridges (Fig. 7 A).

In untreated anaphase cells, TopBP1 localizes to bridge-like structures in 78% of anaphase cells (Fig. 7 A). About 27% of the TopBP1-bound bridges colocalize with PICH UFBs, increasing to 41% after APH treatment, while being largely unaffected by ICRF-193 treatment (Fig. 7 A). The PICH-negative TopBP1 bridges could represent TopBP1 localization at the midbody ( Reini et al., 2004). Interestingly, TopBP1 localization appears to be restricted to a smaller region of the PICH-coated UFBs, at which TopBP1 remains even after dissociation of PICH in late telophase (Fig. 6, B and C). To address whether this region contains single-stranded DNA, we followed the localization of RPA relative to

Table 1. Effect of Dpb11 depletion on mitotic leu2 heteroallelic recombination

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Strain</th>
<th>Treatment</th>
<th>Heteroallelic recombination</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Ratea × 10⁻⁶</td>
</tr>
<tr>
<td>Wild type</td>
<td>ML412</td>
<td>–</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Wild type</td>
<td>ML412</td>
<td>dox</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>tetO2-DPB11</td>
<td>ML767</td>
<td>–</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>tetO2-DPB11</td>
<td>ML767</td>
<td>dox</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>ahc1Δ</td>
<td>ML762</td>
<td>–</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>ahc1Δ</td>
<td>ML762</td>
<td>dox</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>ahc1Δ tetO2-DPB11</td>
<td>ML768</td>
<td>–</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>ahc1Δ tetO2-DPB11</td>
<td>ML768</td>
<td>dox</td>
<td>4.1 ± 0.8</td>
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aRecombination rate (events per cell per generation) is presented as the mean ± SD.

bRelative to the wild type without doxycycline.

Because Dpb11 stimulates Mec1 to phosphorylate a number of checkpoint and repair proteins including the Rad53 kinase, we also monitored Dpb11 bridges in mec1Δ sml1Δ and rad53Δ sml1Δ DNA damage checkpoint–defective strains. Interestingly, both mutants exhibited elevated levels of anaphase bridges delay abscission (Fig. 2 F and Fig. 5 E; Mendoza et al., 2009). To test directly whether Dpb11 is required to delay abscission, we monitored abscission after shutting off expression of Dpb11 by addition of doxycycline. Abscission was assessed using endogenously tagged Ina1 ( YLR413W, indicator of abscission 1). Ina1 exhibits plasma membrane localization similar to the PH domain that was originally used to assess abscission ( Mendoza et al., 2009; Fig. S3). Reduction in Dpb11 expression leads to an accumulation of chromatin bridges, a shift from long to short anaphase bridges, and delayed abscission, demonstrating that Dpb11 is not required for the NoCut checkpoint (Fig. 5, D and E). In contrast, disruption of the previously described NoCut checkpoint by deletion of AHC1 or SLK19 suppressed the accumulation of pre-abscission cells after Dpb11 repression (Fig. 5 E). To test the consequence of premature abscission in the Dpb11-depleted cells, we measured spontaneous recombination between two nonfunctional leu2 heteroalleles in diploid cells. Whereas ahc1Δ or depletion of Dpb11 individually increased interhomologue recombination mildly, the combination of Dpb11 depletion and disruption of the NoCut checkpoint led to a synergistic increase in HR (Table 1).

High levels of Sml1, indicating that a bona fide DNA damage checkpoint has not been activated. In contrast, Rad52- and Hoechst-positive Dpb11 bridges activate the DNA damage checkpoint, leading to Sml1 degradation (Fig. 5, B and C). Although only chromatin bridges activate the DNA damage checkpoint, both types of anaphase bridges delay abscission (Fig. 2 F and Fig. 5 E; Mendoza et al., 2009). To test directly whether Dpb11 is required to delay abscission, we monitored abscission after shutting off expression of Dpb11 by addition of doxycycline. Abscission was assessed using endogenously tagged Ina1 ( YLR413W, indicator of abscission 1). Ina1 exhibits plasma membrane localization similar to the PH domain that was originally used to assess abscission ( Mendoza et al., 2009; Fig. S3). Reduction in Dpb11 expression leads to an accumulation of chromatin bridges, a shift from long to short anaphase bridges, and delayed abscission, demonstrating that Dpb11 is not required for the NoCut checkpoint (Fig. 5, D and E). In contrast, disruption of the previously described NoCut checkpoint by deletion of AHC1 or SLK19 suppressed the accumulation of pre-abscission cells after Dpb11 repression (Fig. 5 E). To test the consequence of premature abscission in the Dpb11-depleted cells, we measured spontaneous recombination between two nonfunctional leu2 heteroalleles in diploid cells. Whereas ahc1Δ or depletion of Dpb11 individually increased interhomologue recombination mildly, the combination of Dpb11 depletion and disruption of the NoCut checkpoint led to a synergistic increase in HR (Table 1).

Because Dpb11 stimulates Mec1 to phosphorylate a number of checkpoint and repair proteins including the Rad53 kinase, we also monitored Dpb11 bridges in mec1Δ sml1Δ and rad53Δ sml1Δ DNA damage checkpoint–defective strains. Interestingly, both mutants exhibited elevated levels of anaphase bridges with mec1Δ sml1Δ inducing primarily UFBs and rad53Δ sml1Δ mostly chromatin bridges (Fig. S4), suggesting that Mec1 and Rad53 are also required to suppress the formation of anaphase bridges. In conclusion, Dpb11 ultrafine anaphase bridges do not activate a bona fide DNA damage checkpoint; rather, Dpb11 is required to suppress the formation of chromatin anaphase bridges by HR.
TopBP1 and Dpb11 bind DNA anaphase bridges • Germann et al.

TopBP1 and Dpb11 bind DNA anaphase bridges • Germann et al.

TopBP1 (Fig. 7 B and Video 2). Interestingly, 24% and 45% of TopBP1 bridges colocalize with RPA after APH or ICRF-193 treatment, respectively, whereas RPA and TopBP1 rarely (1%) colocalize in spontaneous TopBP1 bridges (Fig. 7 C). Importantly, 86% and 83% of the RPA foci observed between the separating chromosomes colocalize with TopBP1 after APH or ICRF-193 treatment, respectively. In conclusion, avian TopBP1 colocalizes with PICH UFBs, and this colocalization is increased in response to replication stress. Moreover, we find that a subset of TopBP1 bridges may contain single-stranded DNA.

TopBP1 depletion causes accumulation of chromatin bridges in anaphase

To determine the impact of TopBP1 on anaphase bridges, we constructed a cell line where all three alleles of TopBP1 are tagged with YFP followed by an auxin-inducible degron (AID), TopBP1-YFP-AID (Nishimura et al., 2009). When expressing the F-box transport inhibitor response protein 1 from *Oryza sativa* (OsTIR1) in these cells, addition of auxin (IAA) promotes interaction between OsTIR1 and TopBP1-YFP-AID, resulting in polyubiquitylation of the AID degron and targeting of TopBP1-YFP-AID for degradation by the proteasome (Nishimura et al., 2009). Depletion of TopBP1 by addition of IAA 30 min before anaphase led to an induction of chromatin bridges and a decreased frequency of UFBs during anaphase (Fig. 7 D). Moreover, depletion of TopBP1 completely inhibited the formation of long PICH-coated UFBs (Fig. 7 F). In fact, PICH UFBs that extend beyond 5 µm are always (98%) bound by TopBP1 (Fig. S5 A). This effect does not appear to be caused by replication stress because APH treatment does not affect the length distribution of PICH-coated bridges (Fig. 7 G), nor can we detect any DNA synthesis during the last 30 min before anaphase entry as based on EdU incorporation, indicating that bulk DNA replication is completed at this stage (Fig. S5, B and C).

To test whether the effect of TopBP1 depletion on DNA bridges is due to its role as an activator of ATR (Kumagai et al., 2006), we treated cells with an ATR inhibitor (ATRi) before anaphase onset (Toledo et al., 2011). Similar to depletion of TopBP1-YFP-AID, treatment with ATRi increased chromatin bridges (Fig. 7 E). However, contrary to depletion of TopBP1-YFP-AID, treatment with ATRi increased the amount of TopBP1-bound UFBs and induced a shift from short to long PICH-coated UFBs (Fig. 7 F). In conclusion, avian TopBP1 colocalizes with PICH UFBs, and this colocalization is increased in response to replication stress. Moreover, we find that a subset of TopBP1 bridges may contain single-stranded DNA.

**Figure 6. Recruitment of TopBP1 to anaphase bridges is evolutionarily conserved.** (A) PICH coats hH2B- and Hoechst-negative UFBs in DT40 cells. Cells (RTP82) expressing PICH-YFP from its endogenous promoter and randomly integrated hH2B-mCherry were stained with Hoechst. Arrowhead indicates a UFB. (B) PICH and TopBP1 colocalize at a subset of UFBs. Cells (RTP149) express TopBP1-YFP and PICH-TFP from their endogenous promoters and hH2B-mCherry. Yellow arrowhead indicates the site of PICH and TopBP1 colocalization at a UFB. Blue arrowhead indicates a PICH-coated UFB without TopBP1. (C) PICH dissociates from UFBs before TopBP1 to form transient sister foci at the termini of the UFB as the cell progresses through anaphase and telophase. Representative time-lapse image sequence of cells (RTP151) expressing TopBP1-YFP-AID and PICH-TFP from their endogenous promoters and randomly integrated OsTIR1 and hH2B-mCherry. Arrowheads indicate TopBP1 structures.
Figure 7. **TopBP1 is required for timely resolution of anaphase bridges.** In all experiments, quantification was performed on the basis of time-lapse microscopy with an imaging frequency of 2 min for 30 min. Exponentially growing cells were monitored from anaphase through telophase, and bridges were scored. The maximum number of bridges visible at one time point was noted as representative for the entire mitosis of a given cell. Asterisk indicates significant differences from the untreated (P < 0.05); error bars represent 95% confidence intervals. The number of cells analyzed is indicated (n).

(A) TopBP1 and PICH colocalizing UFBs are induced by DNA replication stress but not topological stress. Cells expressing TopBP1-YFP-AID, PICH-TFP, OsTIR, and hH2B-mCherry (RTP151) were treated with 0.4 µM APH for 24 h, 0.5 µM ICRF-193 for 30 min, or 0.0125% DMSO (vol/vol, untreated) for 24 h before imaging. (B) A subset of TopBP1 bridges colocalizes with RPA. Cells (RTP156) express TopBP1-YFP, RPA1-CFP, and hH2B-mCherry. Yellow, blue, and green arrowheads indicate TopBP1, RPA1, and colocalizing bridges, respectively. (C) Colocalization of TopBP1 and RPA bridges is induced by both DNA replication stress and topological stress. Cells expressing TopBP1-YFP, RPA-CFP, and hH2B-mCherry (RTP156) were treated with 0.4 µM APH for 24 h, 0.5 µM ICRF-193 for 30 min, or 0.0125% DMSO (vol/vol, untreated) for 24 h before imaging. (D) Depletion of TopBP1 leads to a reduction of UFBs and induction of chromatin bridges. Cells expressing TopBP1-YFP-AID, PICH-TFP, and hH2B-mCherry with OsTIR present (RTP151) or absent (RTP177) were treated with 500 µM IAA or 0.2% ethanol (vol/vol, untreated) for 30 min before imaging. After incubation with IAA for 30 min, the level of TopBP1-YFP-AID fluorescence had decreased below detection in the majority of cells. (E) ATR inhibition induces chromatin bridges and colocalizing PICH- and TopBP1-coated UFBs. Cells expressing TopBP1-YFP-AID, PICH-TFP, and hH2B-mCherry with OsTIR present (RTP151) or absent (RTP177) were treated with 500 µM IAA or 0.2% ethanol (vol/vol, untreated) for 30 min before imaging. After incubation with IAA for 30 min, the level of TopBP1-YFP-AID fluorescence had decreased below detection in the majority of cells. (F-H) TopBP1 depletion, replication stress, and ATR inhibition affect the length of PICH UFBs. The length distribution of UFBs was quantified in anaphase/telophase cells from panels D (IAA), A (APH), and E (ATRi).
In \textit{Saccharomyces cerevisiae}, hemicatenanes form during DNA replication in a Rad52-independent manner (Lopes et al., 2003; Wellinger et al., 2003) and have been proposed to be resolved by the Sgs1–Top3–Rmi1 (BLM–TopoIII$\alpha$–RMI1–RMI2) complex (Wu and Hickson, 2003). Our finding that the frequency of Dpb11 anaphase bridges dramatically increases in an \textit{sgs1} mutant is indicative of hemicatenes constituting a source for anaphase bridges in otherwise unchallenged cells. The anaphase bridges that accumulate in \textit{sgs1} cells are Hoechst positive and dependent on HR for their formation, which is consistent with overexpression of Rad51 leading to chromatin bridges, and with the report that hemicatenes are converted by Rad51 to recombination intermediates in the absence of Sgs1 (Liberi et al., 2005). Likewise, in BLM-deficient human cells elevated levels of both chromatin bridges and lagging chromosomes as well as PICH bridges are observed (Chan et al., 2007).

Catenanes are formed by the noncovalent intertwining of sister chromatids. The primary decatenating activity of the cell is provided by topoisomerase II. To assess the contribution of catenanes in the formation of anaphase bridges, we took advantage of a temperature-sensitive \textit{top2-1} mutant in yeast (Brill et al., 1987) or the topoisomerase II inhibitor ICRF-193 in DT40 cells. In yeast, mutation of \textit{TOP2} leads to an accumulation of UFBs at the semipermissive temperature and to an increase of both UFBs and chromatin bridges at the restrictive temperature. Similarly, ICRF-193 induces both chromatin and PICH UFBs in DT40 cells, which is consistent with results from human cells, where ICRF-159 was reported to induce BLM bridges in a dose-dependent manner (Chan et al., 2007). Thus, our data indicate that sister chromatid catenanes are a source of UFBs in both yeast and DT40 cells.

UFBs (Fig. 7 H). These data indicate that TopBP1 suppresses the formation of chromatin bridges to ensure proper segregation of chromosomes by a mechanism that only partially overlaps with its role as an ATR activator.

\section*{Discussion}

In this study, we establish budding yeast and the avian DT40 cell line as model systems for studying DNA anaphase bridges. In both yeast and DT40 cells we find that when Dpb11/TopBP1 localizes to UFBs, it facilitates their elongation or stability while it suppresses the formation of chromatin bridges. In yeast, Dpb11 UFBs do not activate a bona fide DNA damage checkpoint as measured by Sml1 degradation; instead, anaphase bridges delay abscission by the NoCut checkpoint, independently of Dpb11. Accordingly, simultaneous disruption of the NoCut checkpoint and depletion of Dpb11 led to a synergistic increase in genome instability as measured by interhomologue recombination. In \textit{Schizosaccharomyces pombe}, which does not appear to have a NoCut checkpoint, the Dpb11/TopBP1 orthologue Cut5 was identified along with \textit{top2} mutants for their \textit{cut} phenotype (Hirano et al., 1986), indicating that Cut5 plays a similar role in \textit{S. pombe} in suppressing or resolving anaphase bridges.

Using the budding yeast and DT40 model systems, we tested the proposed sources of anaphase bridges and their putative mechanisms of resolution (Fig. 8): four different DNA structures have been suggested to form anaphase bridges: (1) hemicatenanes arising during DNA replication, (2) catenanes, (3) unreplicated regions or termination zones, and (4) single- and double-Holliday junctions arising from HR.

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DNA replication stress induced by MMS or HU in *S. cerevisiae* or by APH in DT40 cells also leads to UFBs, as previously reported for human cells and *S. pombe* (Chan et al., 2009; Sofueva et al., 2011). Moreover, MMS treatment and the top-1 mutation were additive for induction of UFBs, indicating that replication and topological stress independently lead to these structures.

The majority of chromatin bridges that we observe in *S. cerevisiae* require RAD51, RAD52, and RAD54, indicating that these structures form through HR. This is further supported by the observation that chromatin bridges are bound by Rad52 and trigger degradation of Sm11, indicating that these structures lead to DNA damage signaling. Moreover, chromatin bridges can be induced by overexpression of Rad51 but not by catalytically inactive Rad51 (rad51-K191A). Thus, our work proposes that HR intermediates are a substantial source of chromatin bridges. In line with this, a number of recent articles report that Holliday junction resolvases including MUS81-EME1 and SLX1-SLX4 act in mitosis to preserve genome stability (Matos et al., 2013; Naim et al., 2013; Szakal and Branzei, 2013; Wyatt et al., 2013; Ying et al., 2013). The notion that UFBs are formed independently of HR is supported by RAD51 knockdown in human cells (Chan et al., 2009; Lakhim Bennani-Belhaj et al., 2010). However, the latter study also reported an increase in BLM-associated chromatin bridges upon knockdown of RAD51, which in the light of our data from yeast could reflect stalled HR due to residual RAD51 levels allowing HR to initiate but not supporting its completion.

Based on these results we propose two mutually exclusive roles for Dpb11/TopBP1 at anaphase bridges: (1) suppressing the formation of chromatin bridges and (2) facilitating chromosome segregation through the extension and stabilization of UFBs. Concerning the first point, it has been shown that Dpb11/TopBP1 stimulates Mec1/ATR kinase activity, which in turn regulates HR at replication structures (Lisby et al., 2004; Meister et al., 2005; Kumagai et al., 2006; Mordes et al., 2008; Chanoux et al., 2009; Pfander and Diffley, 2011), pointing to a model where Dpb11/TopBP1 suppresses the formation of anaphase bridges by facilitating the Mec1/ATR-dependent replication checkpoint, thus inhibiting fork collapse and HR (Fig. 8). This is supported by our finding that direct inhibition of the ATR kinase in DT40 cells leads to an accumulation of chromatin bridges, which partially phenocopies auxin-mediated depletion of TopBP1. We have observed a similar increase in the frequency of chromatin bridges in a yeast *rad53Δ sm1Δ* mutant, whereas a *mec1Δ sm1Δ* mutant primarily exhibited an increase in UFBs. The difference between the *rad53Δ* and *mec1Δ* mutant phenotypes could be rationalized by the reported Mec1-independent activities of Rad53 (Clerici et al., 2001; Schramke et al., 2001; Corda et al., 2005). Concerning the second point, stabilizing bridges to help chromosome segregation, the opposing effects of TopBP1 depletion and ATR inhibition on the length distribution of PICH-coated UFBs in DT40 cells indicate that TopBP1 also has a more direct effect on ultrafine bridges, in addition to activating ATR. Dpb11/TopBP1 remains associated with UFBs until late anaphase and apparently facilitates the extension/stability of long UFBs because PICH UFBs rarely extend beyond 5 μm without being bound by TopBP1. This may be related to the interaction of TopBP1 with topoisomerase IIβ (Yamane et al., 1997), which could facilitate the recruitment of topoisomerase IIβ to sites of catenation allowing for progressive disentangling of intertwined sister chromatids. On the other hand, topoisomerase IIα is believed to perform the bulk of decatenation in human cells and depletion of topoisomerase IIα leads to shortening of the metaphase interkinetochore distance and abnormal persistence of PICH-coated anaphase bridges (Porter and Farr, 2004; Spence et al., 2007; Wang et al., 2010). It is not known whether Dpb11 and Top2 interact in yeast, but their frequent colocalization on UFBs could suggest a potential interaction.

The relationship between UFBs and chromatin bridges remains an important open question. However, in the hundreds of anaphase time-lapse microscopy sequences that we have acquired, we have never observed a chromatin bridge turning into a UFB or vice versa. Hence, the determination for a potential initial DNA structure to develop into a chromatin bridge or a UFB appears to be made before anaphase onset. Moreover, several mutants and genotoxic agents induce both kinds of anaphase bridges, suggesting that processing of the initiating DNA structure and its timing relative to, for example, chromosome condensation, may determine whether an anaphase bridge is chromatinized or not.

### Materials and methods

#### Yeast strains and cell culture

Media and standard genetic techniques to manipulate yeast strains were described previously (Sherman, 2002). All yeast strains used in this study are RAD5 derivatives of W303 (Table S1). DT40 cell culture and transfection were done as described previously (Buerstedde and Takeda, 1991). DT40 cell lines used in this study are listed in Table S2.

#### Construction of yeast plasmids and fluorescent fusion proteins

All plasmids are described in Table S3. Oligonucleotide sequences are available upon request. Unless otherwise noted, fluorescent fusion proteins were constructed as described previously (Lisby et al., 2004; Silva et al., 2012). Plasmids pML96 and pML104 for integrating the NLS-RFP fusion protein into the ML8-9A. The KpnI–EcoRI-digested PCR product was cloned into KpnI–EcoRI-linearized pCR2.1-TOPO vector (Invitrogen). The amplified PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen). Primer pairs were designed to facilitate directional cloning.

#### Generation of DT40 knock-in constructs

DT40 genes were endogenously tagged at their 3' termini. The 3' and 5' arms of the TopBP1-YFP-AID knock-in construct were subcloned from pVHC3. To generate 3' and 5' arms for the PICH-YFP/TFP knock-in constructs, flanking regions of homology immediately 5' and 3' of the PICH stop codon were amplified from DT40 genomic DNA. The AID, YFP, and TFP tags were amplified from pMK43, pEYFP-C1, and pmTurquoise2-N1, respectively. Primer pairs were designed to facilitate directional cloning. The amplified PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen) and coding regions sequenced. As a sequence reference for the 3' and 5' arms of the PICH knock-in constructs, the genomic DNA sequence of Gallus gallus (DT40) was obtained from the National Center for Biotechnology Information (Gene ID: 422135). The BSR, NCR, and...
For assembly of the PICH-TFP/YFP knock-in constructs (pRTP6, pRTP9, and pRTP17), the 3' arm, a BSR/PAC cassette, the 5' arm, and the YFP/TFP tag were individually subcloned into pBlueScript SK+ in the listed order using restriction sites Xbal–NotI, BamHI, KpnI–SalI, and SalI–EcoRI, respectively. For assembly of the TopBP1-YFP-AID knock-in constructs (pRTP14, pRTP15, and pRTP16), the YFP tag, the AID tag, the 3' arm, and a BSR/NEO/PAC cassette were individually subcloned into pVHO3 in the listed order using restriction sites XbaI–NotI, BamHI–BglII, BglII–NotI, and BamHI, respectively.

For assembly of hH2B-mCherry under expression control of the β-actin promoter (pRT23), hH2B-mCherry was subcloned from pH2BmCherry_IREs.neo3 in pExpress using the restriction site HindIII. The β-actin promoter and hH2B-mCherry were subsequently subcloned into pLOX/PURO using restriction site SpeI.

The targeting constructs were linearized with NotI before transfection. Transfectants harboring the PAC, NEO, and BSR resistance genes were selected in the presence of 0.5 µg/ml puromycin, 2 mg/ml G418, and 20 µg/ml blasticidin, respectively. The resistance cassettes were laxed as described previously [Arakawa et al., 2001]. In brief, cell lines were transiently transfected with cDNA encoding the Cre recombinase and subsequently diluted cloned to obtain single colonies. Loss of selection markers was tested by treating the resulting cell lines with puromycin, G418, or blasticidin. Integration of the YFP/TFP/YFP-AID tags at the correct genomic location was confirmed by PCR analysis of genomic DNA.

Microscopy and immunofluorescence

Yeast cells were grown in synthetic complete (SC) medium supplemented with 100 µg/ml adenine (SC-Ade) and processed for fluorescence microscopy as described previously [Eckert-Boulet et al., 2011]. For staining of DNA in live yeast cells, 10 µg/ml of DAPI or 5 µg/ml of Hoechst 33342 (Sigma-Aldrich) was added to the culture 10–30 min before microscopy and washed out with fresh medium immediately before microscopy and imaging at 30°C or 37°C as indicated.

Fluorophores used in yeast were CFP (clone W7; Heim and Tsien, 1996), YFP (clone 10C, Ormø et al., 1996), and RFP (clone yEmRFP; Keppler-Ross et al., 2008).

DT40 cells were imaged at 39°C in RPMI 1640 medium GlutaMAX ( Gibco) supplemented with 2% chicken serum (Gibco), 8% fetal bovine serum (Gibco), 2 mM l-glutamine (Gibco), 55 µM β-mercaptoethanol, 50 U/ml penicillin, and 50 µg/ml streptomycin, and mounted on µ-slides −lysine–coated coverslips for 10 min. Cell fixation, permeabilization, and EdU detection was performed as described in the Click-iT EdU Alexa Fluor 594 imaging kit manual.

Recombination assay

Interchromosomal mitotic recombination between leu2 heteroalleles was measured by growing diploid strain at 25°C overnight in 3 ml SC+Ade medium before plating on SC with or without leucine [Smith and Rothstein, 1995]. Colony-forming units were counted after incubation at 25°C for 3 d. The median frequency for 7–11 trials was used to determine the recombination rate by the method of the median. In brief, the median frequency is divided by the factor r0/m to obtain the recombination rate, where m is the mean number of Leu+ recombination events, which have occurred in the culture, and r0 is the number of events in the trial with the median frequency. The factor r0/m was estimated by Lea and Coulson [1949] and the standard deviation was calculated as m·sqrt(12.7/(2.24 + ln(m))2)/N, where N is the number of trials (Lea and Coulson, 1949).

Statistical methods

For microscopy experiments, the significance of the differences between cell populations was determined by one-tailed Fisher’s exact test. P-values were defined as significant if P < 0.05.

Online supplemental material

Fig. S1 shows that Rla1 binds yeast anaphase DNA bridges. Fig. S2 shows that cells rebud after resolution of anaphase bridges. Fig. S3 shows colocalization of the PH domain and Ina1. Fig. S4 shows that Dpb11 bridges accumulate in mec1a and rad53a mutants. Fig. S5 shows EdU incorporation in DT40 and the length distribution of TopBP1/PICH UBFs. Video 1 shows time-lapse microscopy of TopBP1 and PICH. Video 2 shows time-lapse microscopy of TopBP1 and RPA. Table S1 lists the genotype and source of yeast strains used in this study. Table S2 lists the genotype and source of DT40 cell lines used in this study. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201305157/DC1.

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