

Cohesin still drives homologous recombination repair of DNA double-strand breaks in late mitosis

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Abstract

The cohesin complex maintains sister chromatid cohesion from S phase to anaphase onset. Cohesin also plays roles in chromosome structure and DNA repair. At anaphase onset, the cohesin subunit Scc1 is cleaved to allow segregation in an orderly manner, although some residual cohesin subunits remain to maintain chromosome structure. Efficient DNA double-strand break (DSB) repair by homologous recombination (HR) with the sister chromatid also depends on cohesin. Here, we have tested whether residual cohesin is important during DSB repair in anaphase/telophase (late mitosis). Using the well-established MAT switching system, we first show that HR is molecularly functional in late mitosis, and then show that cohesin is required for its efficiency. During DSBs in late mitosis, the segregated sister loci get closer and have episodes of coalescence, which may favour repair by HR. Here, we also show that these cytological phenotypes also depend on cohesin. Finally, full-length Scc1 returns after DSBs in late mitosis, suggesting that *de novo* widespread cohesin complexes can support these retrograde behaviours.

eLife assessment

This study presents evidence that suggests that the coalescence of sister chromatids induced by global double-strand DNA breaks (DSBs) during late mitosis is mediated by cohesin SMC3. These findings are **valuable** for studying the mechanism of eukaryotic cells to repair DNA during late mitosis. Although the discrete DSB induction system in budding yeast is sound, the strength of evidence is **incomplete** and could be buttressed to better support the major claims and to represent a clear advance with respect to the authors' previous contributions to this field.

Introduction

Cell survival and genome integrity depend on the faithful segregation of chromosomes in anaphase. From the S phase until the anaphase onset, the highly conserved cohesin complex plays an essential role in the structural maintenance of sister chromatids. Cohesin is a multiprotein complex composed in yeast of Smc1, Smc3, Scc1 (also known as Mcd1), Scc3 and Pds5 subunits (Hirano, 2000; Michaelis *et al.*, 1997; Sjögren and Nasmyth, 2001). At the core of the complex, Smc1, Smc3 and Scc1 form a heterotrimeric ring that embraces and holds the replicated sister chromatids together and well-aligned until reaching G₂/M (Díaz-Martínez *et al.*, 2008; Koshland and Guacci, 2000; Laloraya *et al.*, 2000; Michaelis *et al.*, 1997; Strunnikov *et al.*, 1993). Cohesin subunits are loaded onto chromosomes in G₁ by the Scc2-Scc3 complex (Ciosk *et al.*, 2000). When cells enter S phase, the acetyl-transferase Eco1 acetylates Smc3, which inhibits the ATPase activity of Smc1-Smc3 heads and prevents the opening of the Smc3-Scc1 interface (Çamdere *et al.*, 2015; Chan *et al.*, 2012; Huber *et al.*, 2016; Murayama and Uhlmann, 2015; Ström *et al.*, 2007; Ünal *et al.*, 2007). This embraces the nascent sister chromatids and establishes cohesion (Uhlmann and Nasmyth, 1998). Once sister chromatids are ready for segregation, the APC^{Cdc20} (Anaphase Promoting Complex associated with its regulatory cofactor Cdc20) initiates anaphase by degrading securin/Pds1, so that the separase/Esp1 becomes active. Separase then cleaves the Scc1 subunit by proteolysis, releasing sister chromatids from cohesion (Cohen-Fix *et al.*, 1996; Michaelis *et al.*, 1997; Nasmyth and Haering, 2009; Uhlmann *et al.*, 1999; Yamamoto *et al.*, 1996). The fragmented Scc1 is then rapidly degraded (Rao *et al.*, 2001), while a pool of Smc1-Smc3 dimers appears to remain through anaphase (Renshaw *et al.*, 2010; Tanaka *et al.*, 1999). This pool however becomes loose as Smc3 is deacetylated by Hos1 in anaphase (Chan *et al.*, 2012; Huber *et al.*, 2016; Murayama and Uhlmann, 2015). Interestingly, some cohesin-dependent cohesion remains at chromosome arms during anaphase, suggesting that residual cohesin is present despite separase activation (Renshaw *et al.*, 2010).

In addition to its role in sister chromatid cohesion, cohesin has also been involved in chromosome structure and DNA repair. On the one hand, cohesin can hold two DNA segments within the same chromatin forming an extruded loop (Lazar-Stefanita *et al.*, 2017; Schalbetter *et al.*, 2017). On the other hand, DNA double strand breaks (DSBs) produce the so-called damage-induced cohesion (DI-cohesion) (Kim *et al.*, 2010). Following DNA damage, cohesin is recruited and accumulated both along 50 – 100 kb surrounding the DSB site and genome-wide (Ström *et al.*, 2004; Ünal *et al.*, 2004). The cohesin recruitment creates a firm anchoring of two well-aligned sister chromatids, which in turn facilitates DSB repair by homologous recombination (HR) (Hou *et al.*, 2022; Phipps and Dubrana, 2022). In this regard, DNA damage kinases such as Mec1 and Chk1 phosphorylate Scc1 to initiate the cohesion establishment in post-replicative cells (Heidinger-Pauli *et al.*, 2008).

HR is a DSB repair mechanism that uses a homologous template for restoring the original broken DNA. HR is highly reliable when the correct template is used, which in mitotic cells is the sister chromatid. Hence, HR is the preferred repair mechanism from S to G₂/M, when the sister chromatid is available in close proximity (Langerak and Russell, 2011; Mathiasen and Lisby, 2014; Symington *et al.*, 2014). Before DNA replication, in G₁, the alternative error-prone non-homologous end joining (NHEJ) is used instead. Cells coordinate the choice of either NHEJ or HR based on the activity of cyclin dependent kinase (CDK). Low CDK keeps cells in G₁ and favours NHEJ, whereas high CDK is present in S and G₂/M and activates HR.

Noticeably, there is a paradox in the link between high CDK and HR. In the cell cycle window that spans from anaphase to the telophase-to-G₁ transition (we will refer to this window as late mitosis), high CDK is set to favour HR despite a sister chromatid is not in proximity (Machín and Ayra-Plasencia, 2020). In a previous study, we showed that sister loci can indeed move closer and coalesce and that HR still appears important for DSB survival in late mitosis (Ayra-Plasencia

and Machín, 2019 [↗](#)). Considering these observations, a second paradox arises, how can sister loci coalesce and HR be that important in the absence of cohesin? We have addressed such a paradox here.

Results and Discussion

The HO-mediated DSB is resected efficiently in late mitosis

In a previous report, we showed from both genetic and cytological points of view that HR was still active in late mitosis. In this work, we applied molecular methods to determine whether HR can occur in late mitosis. To this aim, we followed the DSB processing and repair of a single DSB generated by the inducible HO endonuclease, which cuts the HO cutting site (*HOcs*) at the *MAT* locus in chromosome III. The DSB was generated once cells were arrested in late mitosis (late anaphase/telophase) through the thermosensitive *cdc15-2* allele. Cdc15 is a mitotic kinase that drives telophase-to- G_1 transition (Machín and Ayra-Plasencia, 2020 [↗](#)).

We first tested whether the HO-induced DSB is efficiently resected into 3' ssDNA tails in late mitosis. Resection is necessary to form the protruding 3' nucleofilaments that invade the donor homologous sequence to restore the break by HR (Peng *et al.*, 2021 [↗](#); Symington, 2016 [↗](#)). To confirm and quantitate the formation of ssDNA flanking the *HOcs*, we used a qPCR approach whereby primers can only amplify a target DNA cut with the restriction enzyme *StyI* if it has been rendered single-stranded by resection (Fig 1a, b [↗](#)) (Gnügge *et al.*, 2018 [↗](#); Zierhut and Diffley, 2008 [↗](#)). We used a strain carrying deletions for the *HML* and *HMR* loci, the two ectopic homologous templates for HR with the *MAT* locus. In this manner, the normal HR flow from resection to invasion is blocked, which boosts ssDNA detection. For the controlled induction of the HO endonuclease, we ectopically introduced the encoding gene under the control of a promoter that responds to β -estradiol (Gnügge and Symington, 2020 [↗](#)). Two different locations downstream of the *HOcs* were monitored to determine both the initial resection (0.7 Kb from *HOcs*) and the kinetics further away (5.7 Kb). In addition, cells arrested in either G_2/M (Nz) or late mitosis (*cdc15-2*) were directly compared to each other. Cells were first arrested for 3 h, the HO was induced afterwards, and samples were taken every hour during a 4 h time course while sustaining the HO induction (Fig 1c [↗](#)). As expected, cells in G_2/M efficiently resected the *HOcs* DSB. The time to reach half of the maximum possible resection ($t_{1/2}$) was ~ 1 h at 0.7 Kb and ~ 2.5 h at 5.7 Kb from the DSB, respectively. Resection in late mitosis was only slightly delayed at 0.7 Kb ($t_{1/2} \sim 1.5$ h), whereas the delay was more pronounced at 5.7 Kb ($t_{1/2} \sim 3.5$ h). Overall, resection in late mitosis appears almost as efficient as in G_2/M . The slight delay may be due to either the fact that CDK activity in late mitosis (though still high) is slightly inferior to that at G_2/M , or the different conditions of arrests (Nz at 25 °C vs. just 34 °C). Together with the samples for the qPCR, samples for Western blot of Rad53 were taken. Rad53 is an effector kinase of the DNA Damage Checkpoint (DDC) that gets activated by hyperphosphorylation after being recruited to the 3' ssDNA overhangs (Branzei and Foiani, 2006 [↗](#)). In agreement with the resection profile (Fig 1c [↗](#)), Rad53 hyperphosphorylation also occurred in late mitosis, although with ~ 1 h delay (Fig 1d [↗](#)).

HR can still switch the *MAT* locus in late mitosis

Once resection begins, cells are normally committed to recombine because long ssDNA fragments do not work as efficient substrates for NHEJ. In our haploid strain, HR at the *MAT* locus after the HO-driven DSB implies the gene conversion from the *MATa* to the *MAT α* allele, whereas repair by NHEJ results in reconstitution of the *MATa* allele (Fig 2a [↗](#)) (Yamaguchi and Haber, 2021 [↗](#)). This gene conversion can be detected by Southern blot because of a restriction fragment length polymorphism for *StyI*. Cells that are in G_1 can barely repair the *HOcs* DSB, whereas those in G_2/M do so through HR (Ira *et al.*, 2004 [↗](#)). To check whether HR was efficient at the *HOcs* DSB in late mitosis, we performed an experimental setup similar to the one shown above, but with two

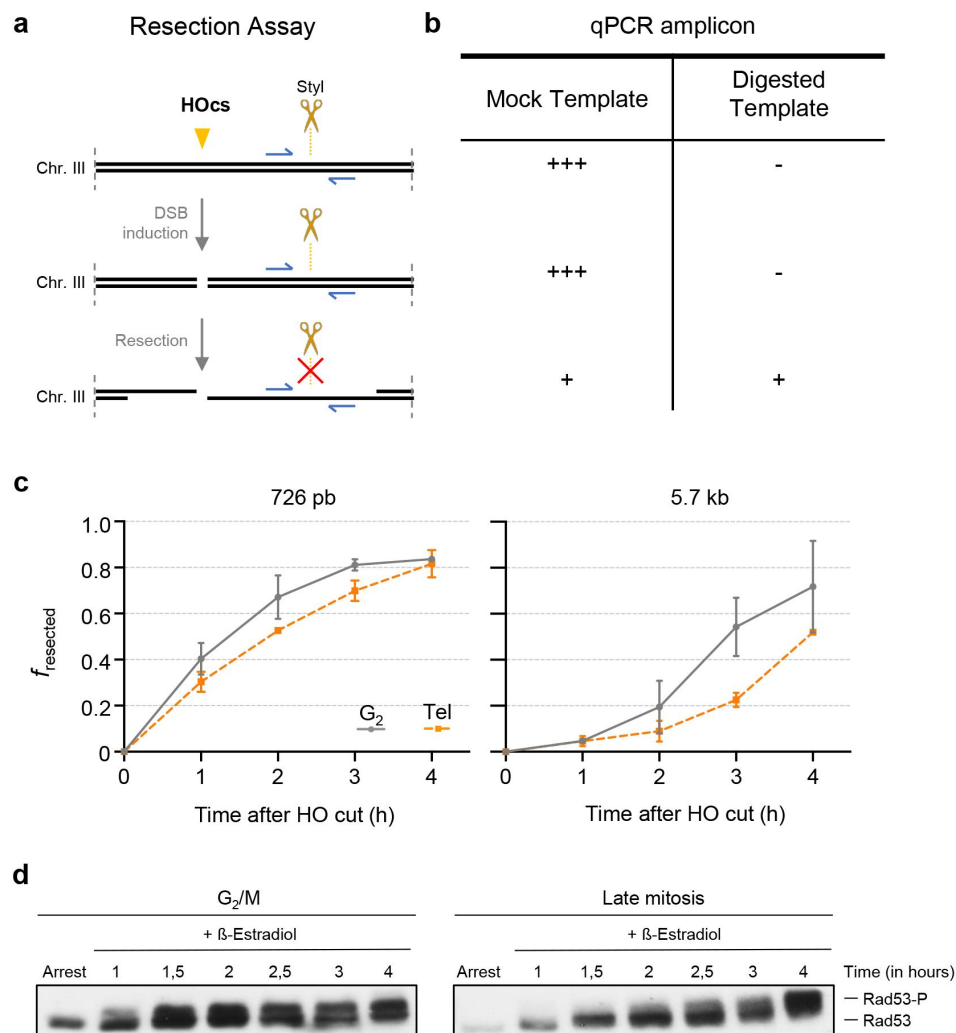


Figure 1.

Resection in late mitosis is almost as efficient as in G₂/M.

(a) Schematic representation of the HO resection assay. Primers (blue arrows) are designed to amplify a sequence that contains a *StyI* target site adjacent to the HO cutting site (HOcs). When this restriction enzyme is used on the extracted genomic DNA, amplification is inhibited. If resection extends beyond the *StyI* site, *StyI* does not cut and the primers can amplify. (b) Summary table of the amplification yield obtained after the *StyI* digestion for each situation (mock control vs. HO induction). (c) Charts depicting the resection kinetics for two different amplicons located at 726 bp and 5.7 kb downstream the HOcs break (mean $\pm$ sem, $n=3$; f_{resected} is the proportion of resected DNA). (d) Representative Western blots against Rad53 to follow the sensing of DNA damage detection in G₂/M versus late mitosis.

important differences. On the one hand, the strain bears the wild type *HML* locus, so that HR can be completed if it is called upon; and on the other hand, a just a pulse of HO induction was applied (1 h with β -estradiol only), so that there is room for the complete repair of all generated DSBs. Samples were taken at the time of the arrest, after the pulse, and all through the repair window. We confirmed by Western blot that HO (HO tagged with the Flag epitope in this case) is produced after the pulse, and rapidly degraded afterwards (**Fig 2b**, HO-Flag blot). Likewise, Rad53 hyperphosphorylation confirmed that the *HOcs* DSB has been correctly sensed (**Fig 2b**, Rad53 blot). The Southern blot showed that (i) most *MATa* locus in the cell population has been efficiently cut after the HO pulse (**Fig 2c** and **d**; decrease of the *MATa* band intensity and rise of that of the HO cut); and (ii) most DSBs are repaired afterwards exclusively through HR (**Fig 2c** and **d**; drop of the HO cut band intensity and rise of that of the *MATa*). No signs of DSB by NHEJ were observed (the remaining *MATa* band stays constant throughout).

Overall, we concluded that HR is the chosen repair pathway in late mitosis. This is in agreement with predictions based on CDK activity and also fits well with the genetic and cytological data we have obtained before (Ayra-Plasencia and Machín, 2019; Machín and Ayra-Plasencia, 2020). By contrast, this emphasizes the paradoxes of having HR in the context of mostly segregated sister chromatids and without the cohesin complex holding them together. The first paradox was at least partly solved in our previous report as we observed that sister loci can rejoin after DSBs (Ayra-Plasencia and Machín, 2019). We have now explored the second paradox further as DI-cohesion appears important for efficient DSB repair by HR (Hou *et al.*, 2022; Phipps and Dubrana, 2022).

The MAT switching in late mitosis depends on cohesin

Smc1 and Smc3 subunits remain attached to chromatin after segregation (Garcia-Luis *et al.*, 2022; Renshaw *et al.*, 2010; Tanaka *et al.*, 1999). In cells arrested in *cdc15-2*, the Smc1-Smc3 pool mostly resides at centromeres where it brings together adjacent regions in both chromosome arms (Garcia-Luis *et al.*, 2022). Of note, this configuration could favour the physical interaction of the *MAT* and the *HML* loci, which may in turn promote a rapid and efficient HR-driven MAT switching. Thus, we tested whether MAT switching was dependent on this residual centromeric cohesin. To this aim, we tagged the Smc3 subunit with the auxin-mediated degron system (*aid**) in the strain that expresses HO from the β -estradiol promoter.

To check that the system was functional, a serial dilution spot assay was carried out (**Fig. 3a**). The strain expressing Smc3-*aid** was plated on YPDA and YPDA plus 8 mM of the auxin indole-acetic acid (IAA). Alongside, we also plated a derivative where we had removed the preceptive F-box protein *OsTIR1*, which forms the functional ubiquitin ligase responsible for targeting the *aid** for degradation upon IAA addition (Morawska and Ulrich, 2013). Since cohesin is an essential complex for vegetative growth, the lack of growth in the *SMC3-AID* OsTIR1* strain strongly points out that Smc3-*aid** is degraded efficiently.

Next, we used this strain to degrade Smc3 after cells have been arrested in late mitosis and before the HO-mediated DSB (**Fig. 3b-d**). In our experimental setup, a pool of Smc3 was clearly present at the *cdc15-2* arrest (**Fig. 3b**). This pool was acetylated at lysine residues K112 and K113, further suggesting that it is chromatin-bound (Rolef Ben-Shahar *et al.*, 2008; Unal *et al.*, 2008). After IAA addition, there was a clear decline in Smc3-*aid** levels. After 1 h in IAA, a 1 h pulse of HO expression was triggered, which allowed us to follow both DSB generation and its subsequent repair without residual cohesin (**Fig. 3c,d**). More than 90% of the Smc3-*aid** had disappeared by the time the HO promoter was shut down.

Contrary to what is seen in the wild type, the Smc3 absence drastically delayed gene conversion from *MATa* to *MATa* (**Fig. 3c,d**). Even though the cut efficiency without Smc3 is lower than in the wild type (~50 % vs. ~70 %, respectively), wild type cells performed HR with ~80 % efficiency (considering the HO cut band as 100 %), whereas Smc3-depleted cells only repaired to *MATa* in

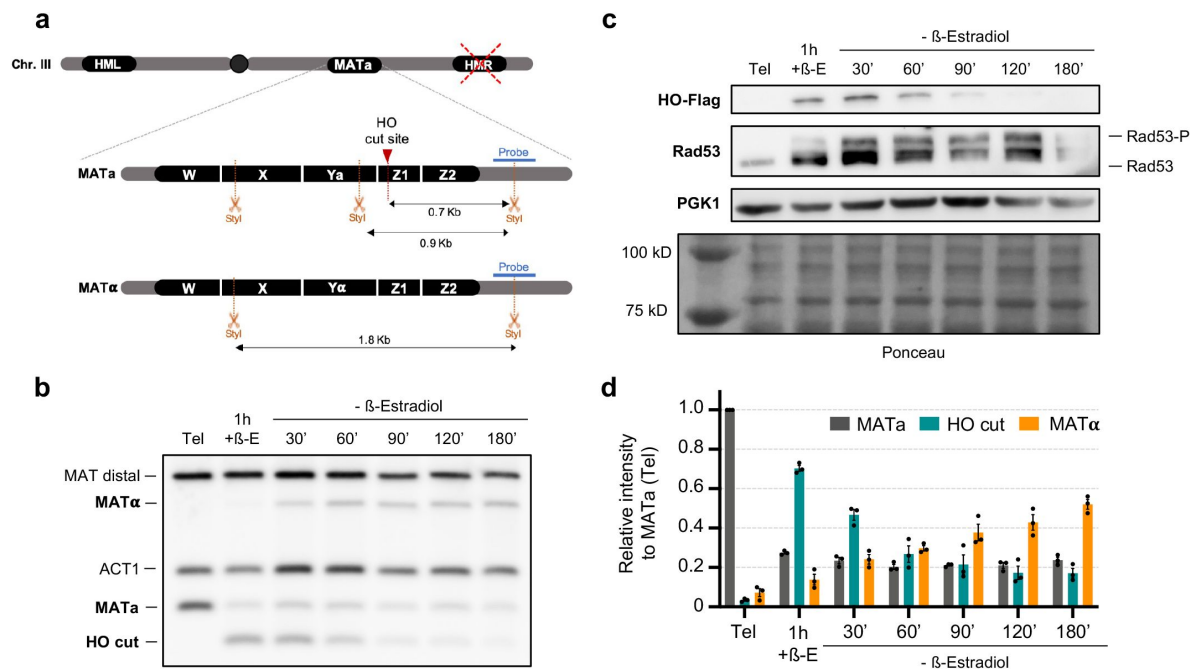


Figure 2.

Yeast cells use HR to repair the HO DSB in late mitosis.

(a) Schematic of the fragments obtained after a *StyI* digestion for both *MATa* and *MATα* sequences. The probe to detect the fragments by Southern blot is in blue. When the *MATa* locus is intact, the digestion gives rise to a 0.9 kb fragment. The HO cutting site (*HOcs*) is located within the *StyI*-digested *MATa* locus. Thus, the HO-driven DSB shortens the fragment to 0.7 kb. HR leads to a gene conversion to *MATα*, which results in the loss of a *StyI* restriction site and a new fragment of 1.8 kb. (b-d) Cells were first blocked in the *cdc15-2* arrest at 34 °C for 3 hours (Tel). Then, the HO-mediated DSB was generated by adding 2 μM β-estradiol. After 1 hour, the β-estradiol was washed away, and samples were taken to monitor the repair for 3 hours. (b) Representative Western blot analyses for HO induction and subsequent degradation (tagged with Flag epitope) and the DSB sensing through Rad53 hyperphosphorylation. A PGK1 western blot served as a housekeeping and Ponceau-S staining of the membrane as a loading control for all lanes. The leftmost lane in the Ponceau-S corresponds to the protein weight marker. (c) Representative Southern blot for the MAT switching assay in late mitosis. Alongside the MAT probe, a second probe against the *ACT1* gene (1.1 kb fragment) was included for normalization. The MAT probe also recognizes an allele-independent MAT distal fragment (2.2 kb). (d) Quantification of relative band intensities in the MAT switching Southern blots (mean ± sem, n=3). Individual values were normalized to the *ACT1* signals. Then, every lane was normalized to *MATa* at the arrest. Tel: Telophase. +β-E: β-estradiol addition.

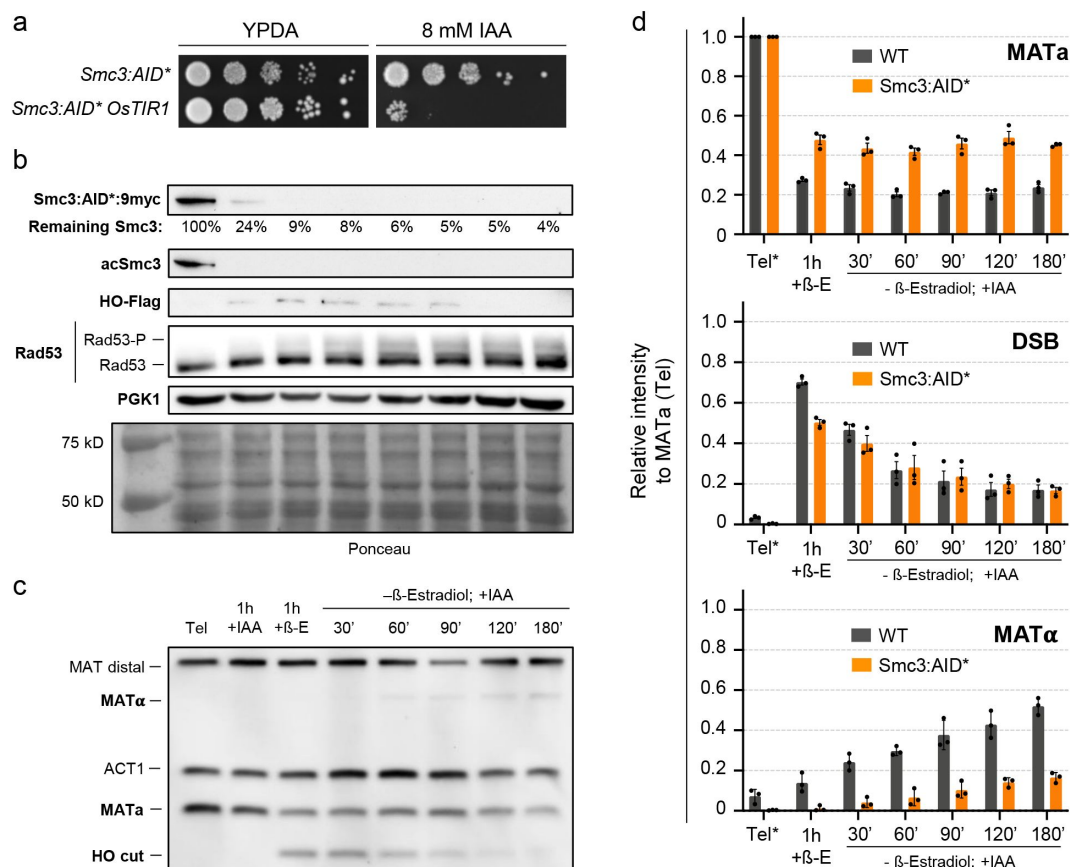


Figure 3.

Smc3 degradation delays HR at the *MAT* locus in late mitosis.

(a) Serial dilution spot assay for the *SMC3:AID** derivative of the strain used in Figure 2. The effect of Smc3-aid* degradation was tested in the presence of 8 mM IAA. The same strain without the ubiquitin-ligase *OsTIR1* was also used as the control. (b-d) Cells were treated as in Figure 2 but including a 1 h IAA step between the *cdc15-2* arrest and the induction of HO. IAA was then maintained throughout the experiment. (b) Representative Western blot as in Figure 2b but also including Smc3-aid* decline after IAA addition. (c) Representative Southern blot for the MAT switching assay after degrading Smc3-aid* in late mitosis. (d) Quantification of relative band intensities for MAT switching Southern blots (mean $\pm$ sem, n=3). Values were normalized as in Figure 2d. The MAT switching in the wild type strain is represented alongside for comparison. Tel: Telophase. +β-E: β-estradiol addition. +IAA: Indole-acetic acid.

~35 %. Also, the detection of DNA damage through Rad53 phosphorylation seems fainter and slightly delayed (**Fig. 3b**). Of note, It was previously reported that cohesin was not that important for the MAT switching in G₂/M-arrested cells, despite it being essential for efficient post-replicative DSB repair (Ünal *et al.*, 2004). One possible explanation for the discrepancy with our MAT switching results may reside in the different experimental setup, i.e. auxin-induced degradation of Smc3 versus Scc1 inactivation using a thermosensitive allele. Alternatively, the Smc1-Smc3 dimer by itself could be sufficient for cohesin-dependent DSB repair. Finally, DI-cohesion in G₂/M is, perhaps, not such important for bringing the *MAT* and *HML* locus together since sister chromatids can rely on other cohesin-independent linkages, such as catenations; a scenario that might not be present in late mitosis.

Scc1 becomes stable after DSBs in late mitosis

At the anaphase onset, the separase/Esp1 cleaves Scc1, opening the cohesin ring and releasing sister chromatids from cohesion. Thereafter, Scc1 needs to be translated *de novo* since cleaved fragments are unstable and degraded by the proteasome (Rao *et al.*, 2001). However, it is known that activation of Esp1 can be blocked by DDC kinases (Yam *et al.*, 2020). In addition, DDC kinases also phosphorylate Scc1 to facilitate DI-cohesion at a post-replicative stage (Heidinger-Pauli *et al.*, 2008). Thus, it is feasible that DSB generation in late mitosis can stabilize *de novo* Scc1 as well as rendering it cohesive.

To know how Scc1 behaves after DSBs in late mitosis, a strain unable to repair the HO-mediated DSB at the *MAT* locus ($\Delta hml \Delta hmr$ double mutant) but bearing Scc1 tagged with three tandem copies of the *myc* epitope was first blocked in telophase at 34 °C for 3 hours. Then, the culture was divided into three. The first one served as a mock control, whereas the others were used to generate a single (2 μ M β -estradiol) and multiple (10 μ g · mL⁻¹ phleomycin) DSBs. Incubation of subcultures was prolonged at 34 °C for 2 extra hours. The presence or absence of Scc1-3myc was then checked by Western blot. As expected, Scc1 band was detected for both cycling cells and those blocked in G₂/M. The Scc1 signal disappeared almost entirely when cells were arrested in late mitosis and remained low in the mock control two hours later. Surprisingly, full-length Scc1 was restored after single and multiple DSBs generation (**Fig. 4a**). This finding suggests that the cohesin complex could be newly formed and loaded onto the breaks, promoting the DI-cohesion and easing the recombinational coalescent events.

Sister loci approximation and coalescence after DSBs in late mitosis also depend on cohesin

Considering the above results on Smc3-dependent HR and Scc1 reappearing after DSBs in late mitosis, we next studied whether cohesin was needed for the remarkable phenotypes we described before under the microscope. The most striking phenotype was the partial regression of sister chromatid segregation, which included back migration and coalescence of sister loci in a single cell body of the dividing cell (Ayra-Plasencia and Machín, 2019). To this aim, the same strain we used before for these studies was transformed to obtain Smc3-aid*. The strain carries several *tetO* sequences at the telomeric region of the right arm of chromosome XII (cXIIR-Tel), as well as the TetR-YFP as the fluorescent reporter for both sister telomeres. The cXIIR-Tel is the last to segregate in anaphase (Machín *et al.*, 2005); hence, back migration and coalescence of these sister loci are bona fide signs of regressed anaphase (Ayra-Plasencia and Machín, 2019). Cells arrested in late mitosis were submitted to Smc3 degradation and subsequent generation of DSBs as above. Again, Western blot analyses showed that cells degraded ~ 80 % of Smc3 after 1 hour in IAA, and subsequent generation of DSBs with phleomycin triggered Smc3-independent Rad53 phosphorylation at similar levels than the control without IAA, positioning any cohesin role downstream of the DDC (**Fig. 4b**). Cells that were not treated with IAA but were having DSBs reverted segregation of cXIIR-Tel in ~ 13 % of the telophase-blocked cells [p<0.0001 relative to categories quantified at the arrest] (**Fig. 4c**). As we found before (Ayra-Plasencia and Machín,

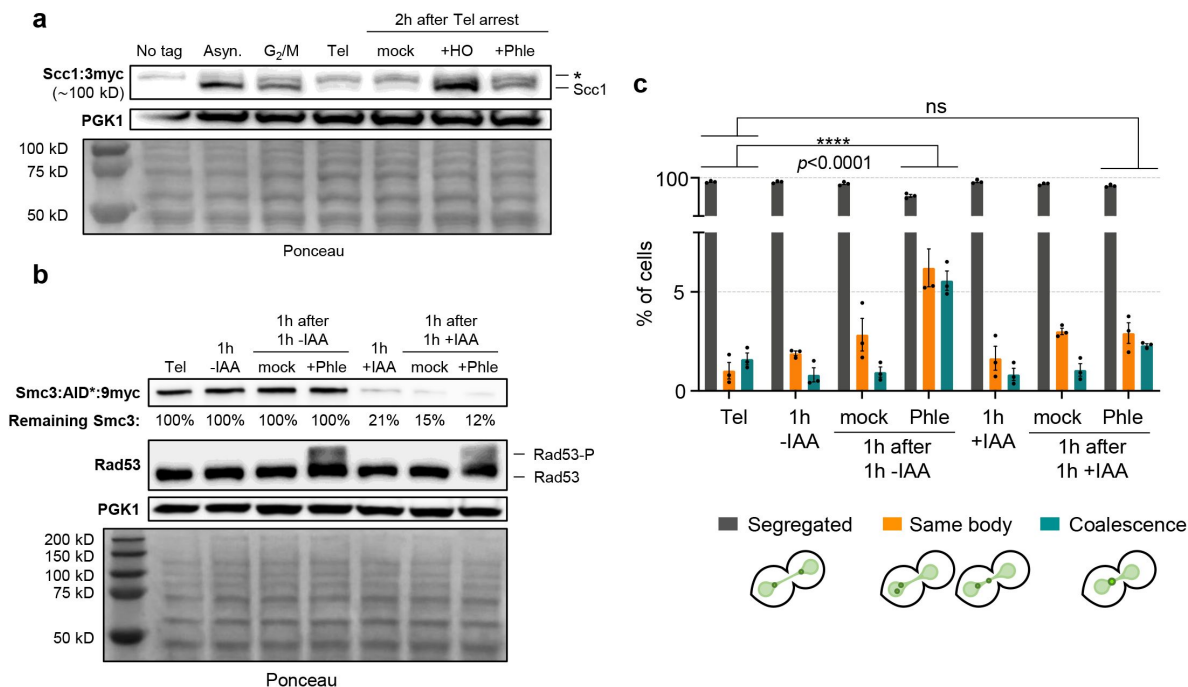


Figure 4.

Scc1 returns after DSBs in late mitosis and the absence of Smc3 inhibits sister telomere coalescence.

(a) Western blot against Scc1-3myc (detected with an α -myc antibody). This experiment compares Scc1-3myc levels in an asynchronous, G₂/M- and telophase-blocked cultures. Cells arrested in telophase were further subdivided into three subcultures and treated as indicated for another two hours (mock, phleomycin, and HO endonuclease). The leftmost lane is a control strain for Scc1 without the 3myc epitope tag. PGK1 protein served as a housekeeping. Ponceau-S staining is also shown as a loading control. Asyn.: Asynchronous. Tel: Telophase. +Phle: 10 mg·mL⁻¹ phleomycin. +HO: 2 μ M β -estradiol. *: Unspecific band detected by the α -myc antibody just over the Scc1-3myc signal. (b, c) A *SMC3:AID** strain that reports the segregation status of the chromosome XII right telomere (cXIIr-Tel) was used for this regressed anaphase assay. Cells arrested in late mitosis were divided into two. One subculture was treated with 8 mM IAA for 1 h. Then, each subculture was, in turn, divided into two, one serving as a mock control and the other was treated with 10 μ g·mL⁻¹ phleomycin for an extra hour. Samples were taken at the indicated time points for Western blotting and microscopy. (b) Representative Western blot. Smc3-aid* was monitored using an anti-mini-AID monoclonal antibody. Rad53 hyperphosphorylation was also monitored as a control to confirm DNA damage after phleomycin addition. PGK1 was used as the housekeeping control to quantify the remaining Smc3 signal. Ponceau-S staining of the membrane is also shown as a loading reference for each lane. (c) Chart representing quantifications of sister telomere segregation status (mean $\pm$ sem, n=3). Cells were classified into three categories depending on cXIIr-Tel location within the elongated nucleus: (i) Segregated, showing two foci well separated in distinct cell bodies; (ii) same body, presenting both sister telomeres in one of the daughter-to-be cells; and (iii) coalescence, only one cXIIr-Tel focus. Statistical significance was calculated through 2-way ANOVA and multiple comparisons analysis. IAA: Indole-acetic acid; Tel: Telophase (i.e. late mitosis); ns: non-significant statistical difference.

2019 [1](#)), ~ 7 % of cells showed two foci in the same cellular body (i.e. back migration), whereas ~ 6 % had coalescence events. In contrast, cells deficient for Smc3 did not show a significant statistical difference from the mock non-DSB situation. This result supports that the cohesin complex is at least essential to promote the passing of chromosomes across the bud neck, which results in back migration and is a prerequisite for the subsequent coalescence.

Conclusions

In this work we have addressed whether HR was molecularly efficient in yeast cells arrested in late mitosis. We have used the well-established MAT switching system and found that HR is indeed working in late mitosis, complementing previous results that pointed in this direction both genetically and cytologically (Ayra-Plasencia and Machín, 2019 [1](#)). In addition, we have addressed that paradox of having HR in a cell mitotic stage with no sister chromatid cohesion. Our results suggest not only that residual cohesin, or at least residual Smc1-Smc3 dimers, play a role in an efficient HR at the *MAT* locus, but also that the partial regression of the anaphase we cytologically observed before is dependent on cohesin. The fact that Scc1, the cohesin subunit that is cleaved and degraded at the anaphase onset, returns in late mitosis after DSB generation, suggests that *de novo* cohesion could be established, perhaps globally. This new cohesin may favour repair by HR with the intact sister of DSBs generated in such a late cell cycle stage. Further research will be needed to confirm whether cohesion is re-established in late mitosis upon DNA damage as well as its whereabouts and consequences for late segregation after DSB repair.

Material and Methods

Strains and experimental conditions

The **table 1** [1](#) contains the *S. cerevisiae* strains used in this work. Genetic backgrounds are either W303 or YPH499. Strain construction was either performed by lithium acetate-based transformation on pre-made frozen competent cells, either by crosses and sporulation (Knop *et al.*, 1999 [1](#)).

Strains were cultured overnight in the YPD medium (10 g·L⁻¹ yeast extract, 20 g·L⁻¹ peptone and 20 g·L⁻¹ glucose) with moderate shaking (180 rpm). The day after, 10-100 µL of grown cells were diluted into a flask containing an appropriate YPD volume and grown overnight at 25 °C again. Finally, the exponentially growing culture was adjusted to OD₆₀₀ = 0.5 to start the experiment.

In general, cells were first arrested in late mitosis by incubating the culture at 34 °C for 3 hours (all strains carry the *cdc15-2* thermosensitive allele). Then, the culture was split in two, one subculture remained untreated (mock), and DSBs were generated in the second one. When a G₂/M arrest was required, 15 µg·mL⁻¹ nocodazole (Nz; Sigma-Aldrich, M1404) was added to the asynchronous culture, which was then incubated at 25°C for 3 h (with a 7.5 µg·mL⁻¹ Nz shot at 2 h). To conditionally degrade the Smc3-aid* variant, 8 mM of the auxin 3-indol-acetic acid (IAA; Sigma-Aldrich, I2886) was added to the arrested cells 1 h prior to generating DSBs.

DSB generation was accomplished by incubating the culture for 1 to 3 hours with either 10 µg·mL⁻¹ phleomycin (random DSBs) or 2 µM of β-estradiol (HO-driven DSBs). For the HOcs DSB, the corresponding strain harbours an integrative system based on a β-estradiol inducible promoter (Ottoz *et al.*, 2014 [1](#)). These strains were constructed by transforming with the pRG464 plasmid (Gnügge and Symington, 2020).

Strain	Relevant Genotype ¹	Origin
FM588	(YPH499) <i>MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 bar1-Δ; ade2-101::TetR:YFP::ADE2; tetO(5.6Kb)::1061Kb-ChrXII::HIS3; cdc15-2:9myc::Hph</i>	F. Machín lab
LSY4319-1C	(W303) <i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 RAD5 Δhml Δhmr; leu2-3::lexO4p:HO:FLAG:CYC1t::ACT1p:LexA-ER-B112-T:CYC1p::LEU2MX</i> (pRG646)	L. Symington lab
FM2450	LSY4319-1C; <i>cdc15-2:9myc::Hph</i>	This work
FM2520	FM2450; <i>SCC1:3myc::HIS3MX</i>	This work
FM2531	FM588; <i>HMLα Δhmr::HIS3MX; leu2-3::lexO4p:HO:FLAG:CYC1t::ACT1p:LexA-ER-B112-T:CYC1p::LEU2MX</i>	This work
FM2672	FM2531; <i>SMC3:AID*:9myc::KanMX</i>	This work
FM2680	FM2672; <i>ura3-52::ADH1p:OsTIR1:9myc::URA3</i>	This work
FM2626	FM588; <i>SMC3:AID*:9myc::KanMX</i>	This work
FM2628	FM2626; <i>ura3-52::ADH1p:OsTIR1:9myc::URA3</i>	This work

¹ Semicolons separate genetic modifications obtained through sequential transformation steps. Intermediate strains are omitted

Table 1.

Strains used in this work.

qPCR for DSB resection

In the resection experiments, qPCR was performed on genomic DNA (gDNA) extracted by the glass beads/phenol method (Hoffman and Winston, 1987 [\[1\]](#)). Experimental details on the resection assay can be found in (Gnügge *et al.*, 2018 [\[2\]](#)). Briefly, each gDNA sample was divided into two and one aliquot was digested with *StyI*-HF (NEB, R3500S). Then, qPCR reactions were mounted using the PowerUp™ SYBR™ Green Master Mix (Thermo Scientific, A25741) and run in a BioRad CFX384 Real-Time PCR instrument (10 µl final volume in 384-well block plates).

Resection was calculated as a normalized fraction (f_{resected}) to that of the HOcs effectively cut by HO (f). These two parameters were calculated by their corresponding formulas (Gnügge *et al.*, 2018 [\[2\]](#)).

Western blot for protein levels and phosphorylation

Western blotting was performed as reported before (Ayra-Plasencia and Machín, 2019 [\[3\]](#)). The trichloroacetic acid TCA method was used for protein extraction. Total protein was quantified in a Qubit 4 Fluorometer (Thermo Fisher Scientific, Q33227). Proteins were resolved in 7.5% SDS-PAGE gels and transferred to PVDF membranes (Pall Corporation, PVM020C099). The membrane was stained with Ponceau-S solution (PanReac AppliChem, A2935) as a loading reference. The following primary antibodies were used for immunoblotting: mouse monoclonal α -HA (1:1,000; Sigma-Aldrich, H9658); mouse monoclonal α -myc (1: 5,000; Sigma-Aldrich, M4439); mouse monoclonal α -Pgk1 (1:5,000; Thermo Fisher Scientific, 22C5D8), and mouse monoclonal α -minioid (1:500; MBL, M214-3). The secondary antibody was a horseradish peroxidase polyclonal goat anti-mouse (from 1:5,000 to 1:10,000, depending on the primary antibody; Promega, W4021). Proteins were detected with the ECL chemiluminescence reagent (GE Healthcare, RPN2232), and visualized in a Vilber-Lourmat Fusion Solo S chamber.

Southern blot for the MAT switching assay

In this case, gDNA was extracted by digesting with 50U lyticase (Sigma-Aldrich, L4025) a cell pellet previously resuspended in 200 µl of digestion buffer (1 % SDS, 100 mM NaCl, 50 mM Tris-HCl, and 10 mM EDTA). Then, the gDNA was phase separated by phenol:chloroform (PanReac AppliChem, A0944), precipitated with ethanol, resuspended in TE 1X with 10 µg·mL⁻¹ RNase A, precipitated again, and resuspended in TE 1X. Then, the gDNA was digested with *StyI*-HF, the restriction fragments separated on a 1.2% low EEOO LS Agarose gel, and finally Southern blotted. Southern blot was carried out by a saline downwards transference onto a positively charged nylon membrane (Hybond-N+, Amersham-GE; RPN303B) (García-Luis and Machín, 2014 [\[4\]](#)). DNA probes against *ACT1* and *MAT* loci were made using Fluorescein-12-dUTP Solution (ThermoFisher; R0101) and the Expand™ High Fidelity PCR System (Roche; 11732641001). Hybridization with fluorescein-labelled probes was performed overnight at 68°C. The following day, the membrane was incubated with an anti-fluorescein antibody coupled to alkaline phosphatase (Roche; 11426338910), and the chemiluminescent signal detected using CDP-star (Amersham; RPN3682). Blots were visualized in a Vilber-Lourmat Fusion Solo S chamber.

For band quantification, each individual band was normalized to the ACT1 signal in the lane. Then, a second normalization to the MATa band at the arrest was performed.

Data representation and statistics

Three types of graphs were used to represent the data: bar charts, marker line graphs and box plots. In box plots, the center line represents the medians, box limits represent the 25th and 75th percentile, the whiskers extend to the 5th and 95th percentiles, and the dots represent outliers. Error bars in all bar and line charts represent the standard error of the mean (sem) of three independent biological replicates. Individual values are also represented as dots in the bar charts.

Graphpad Prism 9 was used for generating the charts and for statistical analysis. Differences between experimental data points were generally estimated using the Mann-Whitney U test for box plots and two-way ANOVA with Tukey post hoc for bar charts.

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Author contributions

J Ayra-Plasencia: Performed all experiments in this work; constructed all strains; prepared the corresponding figures; gave critical insights as to the direction and development of the study; and co-wrote the manuscript.

L Symington: Results shown in **Figures 1** [↗](#) and **2** [↗](#) were generated in her lab; hosted J.A-P. during the generation of these results; gave critical insights as to the direction and development of the study; and was responsible for funding acquisition and project administration.

F Machín: Results shown in **Figures 3** [↗](#) and **4** [↗](#) were generated in his lab; was the supervisor of J.A-P.; gave critical insights as to the direction and development of the study; was responsible for funding acquisition and project administration; and co-wrote the manuscript.

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Reviewer #1 (Public Review):**Summary:**

The cohesin complex maintains sister chromatid cohesion from S phase to anaphase. Beyond that, DSBs trigger cohesin recruitment and post-replication cohesion at both damage sites and globally, which was originally reported in 2004. In their recent study, Ayra-Plasencia et al reported in telophase, DSBs are repaired via HR with re-coalesced sister chromatids (Ayra-Plasencia & Machín, 2019). In this study, they show that HR occurs in a Smc3-dependent way in late mitosis.

Strengths:

The authors take great advantage of the yeast system, they check the DSB processing and repair of a single DSB generated by HO endonuclease, which cuts the MAT locus in chromosome III. In combination with cell synchronization, they detect the HR repair during G2/M or late mitosis. and the cohesin subunit SMC3 is critical for this repair. Beyond that, full-length Scc1 protein can be recovered upon DSBs.

Weaknesses:

These new results basically support their proposal although with a very limited molecular mechanistic progression, especially compared with their recent work.

- <https://doi.org/10.7554/eLife.92706.1.sa1>

Reviewer #2 (Public Review):**Summary:**

The manuscript "Cohesin still drives homologous recombination repair of DNA double-strand breaks in late mitosis" by Ayra-Plasencia et al. investigates regulations of HR repair in conditional *cdc15* mutants, which arrests the cell cycle in late anaphase/telophase. Using a non-competitive MAT switching system of *S. cerevisiae*, they show that a DSB in telophase-arrested cells elicits a delayed DNA damage checkpoint response and resection. Using a degtron allele of SMC3 they show that MAT α -to- α switching requires cohesin in this context. The presence of a DSB in telophase-arrested cells leads to an increase in the kleisin subunit Scc1 and a partial rejoining of sister chromatids after they have separated in a subset of cells.

Strengths:

The experiments presented are well-controlled. The induction systems are clean and well thought-out.

Weaknesses:

The manuscript is very preliminary, and I have reservations about its physiological relevance. I also have reservations regarding the usage of MAT to make the point that inter-sister repair can occur in late mitosis.

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