**DNA synthesis is required for resolution of chromatin bridges in MEN-deficient cells**

To determine if MEN bridges are due to persistent cohesion between replicated sister DNA molecules, we used *cdc15-as1* mutants to inactivate MEN by addition of the ATP analogue 1-NA-PP1, and a ts allele of the kleisin subunit Scc1 to inactivate cohesin. Cells were grown in the presence of 1-NA-PP1 at 25 ºC to inactivate MEN, and were then shifted to 37 ºC to inactivate cohesin. Bridge resolution and cytokinesis were followed using Htb2-mCherry and Myo1-GFP as reporters by time-lapse microscopy. Chromatin bridges persisted in 87% of *cdc15-as1* cells shifted to 37 ºC for 3 hours, in both *SCC1+* and *scc1-ts* backgrounds, indicating that MEN bridges are stable and are not due to persistent sister chromatid cohesion (**Fig. 5a**).

Washout of 1-NA-PP1 led to chromatin bridge disappearance before cytokinesis (monitored with Htb2-mCherry and Myo1-GFP, as above) in the majority of *cdc15-as1* cells, demonstrating that MEN reactivation allows chromatin bridge resolution. This allowed us to test for proteins required for the resolution of chromatin bridges after MEN reactivation. Inactivation of type II topoisomerase in *cdc15-as1 top2-4* cells, by increasing the temperature to 37 ºC before 1-NA-PP1 washout, did not reduce the efficiency of bridges resolution before actomyosin ring contraction (**Fig.** **5b**). Thus, MEN-deficient bridges are not caused by persistent DNA catenations.

In contrast, inactivation of DNA polymerases delta or epsilon caused bridges persisted until cytokinesis in the majority of *cdc15-as1 pol3-ts* and *cdc15-as1 pol2-ts* cells after MEN reactivation, indicating that MEN-deficient bridges require DNA synthesis for their resolution **(Fig. 5c)**. Timely resolution of chromatin bridges in *cdc15-as1* cells after 1-NA-PP1 washout was not impaired by loss of the homologous recombination factor Rad51 (*cdc15-as1 rad51∆*). In contrast, bridge resolution efficiency was reduced in cells lacking the DNA polymerase delta subunit Pol32 (*cdc15-as1 pol32*) **(Fig. 5c** and **S6)**. Pol32 is required for DNA synthesis initiated from DNA double strand breaks or collapsed replication forks, a process known as break-induced replication (BIR) [14](https://paperpile.com/c/ugV5xT/Yv9Q). Thus, our results open the possibility that a fraction of MEN-deficient bridges are resolved by Rad51-independent BIR (discussed in [15](https://paperpile.com/c/ugV5xT/i5qa)). Consistent with DNA synthesis along chromatin bridges in MEN-deficient cells, RPA foci indicative of ssDNA are present in most *cdc15-as1* cells treated with 1-NA-PP1 during anaphase **(Fig. 5d)***.* In summary, complete chromosome segregation is promoted by MEN function and DNA synthesis during anaphase.