Mutations in a partitioning protein and altered chromatin structure at the partitioning locus prevent cohesin recruitment by the yeast plasmid and cause its missegregation

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Supplementary Materials

Interaction of Rep1p mutants with wild type Rep1p

In order to test whether any of the Rep1p mutants constructed in this study were affected in interaction with wild type Rep1p, dihybrid assays were carried out in the Brent and Finley system (2) using experimental protocols described previously (1; Fig. S1A). The common bait in the binary interaction tests was wild type Rep1p fused to LexA repressor. Positive interaction with a Rep1p mutant (fused to acidic patch activation domain) was indicated by colony growth in –His –Trp –Leu plates in the presence of galactose (top row; Fig. S1A) but not dextrose (middle row; Fig. S1A). All of the mutant Rep1 proteins tested here retained their ability to interact with wild type Rep1p. For a subset of the mutants, this inference from the dihybrid analysis was further verified by an in vitro pull down assay using GST-Rep1p and S-peptide tagged Rep1p mutants (Fig. S1B).
Fig. S1. Dihybrid analysis and *in vitro* ‘pull-down’ assays for the interaction between wild type Rep1p and Rep1p mutants. A. In the dihybrid assays, wild type Rep1p fused to the LexA repressor was tested against each of the indicated Rep1p mutants fused to the transcriptional activation domain. The two plasmids containing the bait and prey, respectively, were maintained in the host strain by selecting for the markers they harbored, Trp in one case and His in the other (bottom row). The negative control C1 for the assay was provided by an ‘empty’ vector, in which the activation domain was not fused to Rep1p or a mutant derived from it. The positive control C2 was supplied by wild type Rep1p fused to the activation domain. B. The ‘pull-down’ assays were carried out using fusion proteins expressed in *Escherichia coli*, as detailed earlier (1, 4). A GST-Rep1p (wild type) hybrid protein was used to trap S-tagged wild type Rep1p or the indicated Rep1p variants. ‘I’ refers to the input protein level; ‘P’ stands for the corresponding amount pulled down by the GST-Rep1p trap. GST alone (without fused Rep1p) was not able to pull down wild type Rep1p (left panel).
Association of inappropriately expressed Mcd1p with STB during a cell cycle in a synchronized population: Class I-III Rep1p mutants fail to mediate Mcd1p recruitment at STB

The ChIP results displayed in Fig. 2 of the manuscript using cells in the mid-log phase of growth revealed that representative members of Class I-III Rep1p mutants, unlike wild type Rep1p, were not functional in the recruitment of cohesin at STB. A similar result was obtained when a Class II Rep1p mutant was tested in G1 arrested cells expressing Mcd1p from the GAL promoter (Fig. 3A of the manuscript). To clarify these issues further, we assayed the mutants in cells first arrested in G1 using α factor and then allowed to proceed through the cell cycle in a synchronous fashion (Fig. S2). The time of release from pheromone arrest is indicated as 0 min. in Fig. S2. In contrast to a normal situation, Mcd1p expression, driven by the inducible GAL promoter, was artificially kept on throughout the cell cycle in the present assay. As a reminder, the phenotypes of the Rep1p mutants are as follows. Rep1p (K297Q) is normal in its interaction with Rep2p but not with STB (Class I), whereas Rep1p(Y43A) shows the reverse phenotype (Class II). Rep1p(T32K) has lost both types of interactions (Class III).

The results with wild type Rep1p, depicted in panel A of Fig. S2 over a 90 min. time span, provides a frame of reference for those obtained with the Rep1p mutants arranged in panels B-D. The G1 expressed Mcd1p was associated with STB from 0-30 min. in the presence of wild type Rep1p; however, Mcd1p was absent from the chromosome V cohesin binding site during this period and at 45 min. (compare rows 1 and 2 in A). Both Rep1 and Rep2 proteins were present at STB from 0-30 min. (rows 3 and 4 in A). None of the three proteins could be detected at STB at the 45 min. time point.
(rows 1, 3 and 4 in A). They reemerged at \textit{STB} at 60 min., and continued this association through 75 min. and 90 min. (rows 1, 3 and 4 in A). The PCR signal strengths at the latter two time points were clearly stronger than that at 60 min. A very similar pattern of Mcd1p association was seen at the chromosome V locus during the 60-90 min interval (compare row 2 to rows 1, 3 and 4 in A). The cycling of the Rep proteins at \textit{STB} agrees with a similar outcome documented in Fig. 3B of the manuscript for dextrose grown cells. The timings of this event are not directly comparable between the two experiments because of the longer cell cycle duration in galactose compared to dextrose.

Based on the results with wild type Rep1p (Fig. S2A), the ChIP assays for cells expressing mutant Rep1 proteins were performed at three time points: 0 min. (release from G1 arrest), 45 min. (time of exit of Rep1p and Rep2p from \textit{STB}) and 90 min. (S phase; presence of Mcd1p at \textit{STB} as well as chromosome V). None of the three mutant Rep1 proteins supported the association of Mcd1p with \textit{STB} at 0 min. (G1 phase) or at 90 min. (S phase) (top row, Fig. S2B-D). As expected from the \textit{REP1} panel at the left, Mcd1p was present at the chromosomal site at 90 min. but not at 0 min. or 45 min. (row 2, Fig. S2B-D). Consistent with the phenotypes of the Class I-III Rep1p mutants, Rep1p(Y43A) was detected at \textit{STB} at 0 min. and at 90 min. but not Rep1p(K297Q) nor Rep1p(T32K) (row 3, Fig. S2B-D). By contrast, wild type Rep2p was associated with \textit{STB} at 0 min. and 90 min. regardless of the nature of the mutation (Class I, II or III) present in its Rep1p partner (row 4, Fig. S2B-D).
Fig. S2. Interaction of Mcd1p, wild type Rep1p or Rep1p mutants and Rep2p with STB in synchronized cells continuously expressing Mcd1p from the GAL promoter. The [cir0] host strain contained a galactose inducible copy of the MCD1 gene tagged with the myc epitope. The strain expressed wild type Rep2p from its native promoter. The ChIP assays were carried out in derivatives of this strain expressing wild type Rep1p or the Rep1p mutants indicated above the respective panels by their gene designations. The cell cycle arrest at G1 as well as the release from this arrest was performed in medium containing galactose as the sole carbon source. Antibodies to the myc epitope or to the native Rep proteins were used in immunoprecipitations, as indicated on the right hand side of rows 1-4 from top. WCE refers to ‘whole cell extracts’ that served as positive controls for PCR. As denoted on the left hand side of each row, the primers used were targeted to the STB locus or to a cohesin binding site on chromoosmeV (3).
The above results fully corroborate those presented in Fig. 2 and 3A of the manuscript. First, the Class 1-III Rep1p mutants cannot assist wild type Rep2p in the assembly of cohesin at STB, be it normal assembly during S phase or artificial assembly during G1 due to untimely expression of Mcd1p (row 1, Fig. S2B-D). During G1, Mcd1p is only associated with STB and not with a chromosomal cohesin binding site (compare 0-30 min. lanes in rows 1 and 2 of Fig. S2A). The Rep1 and Rep2 proteins occupy STB in a mutually independent manner, as is also true of their temporary exit from STB during the G1-S window. The Class II mutant Rep1p(Y43A) is present at STB at 0 and 90 min., but not at 45 min., even though it cannot interact with Rep2p (row 3, Fig. S2C). A similar pattern of STB association/exit is displayed by Rep2p even when its partner Rep1p variant cannot interact with STB (row 4, Fig. S2C and D).

References:


   The 2 micrometer plasmid stability system: analyses of the interactions among plasmid- and host-encoded components. Mol Cell. Biol **18**:7466-77.