

## Stm1 interacts with Cdc13, a regulator of the telomere replication in yeast *Saccharomyces cerevisiae*.

N. Hayashi, and S. Murakami

We tried to isolate and characterize the factors interacting with *CDC13* in order to clarify the molecular aspects of telomere replication. Cdc13 interacts with Est1 and DNA polymerase  $\alpha$ , and *cdc13-1*, one of the mutations in *CDC13*, cannot complete the telomere replication at restrictive temperature. This *CDC13* gene, encoding a binding protein with a single G-rich strand at the telomere, is the regulator of telomere replication. In a 2-hybrid screening using *CDC13* as bait, *STM1* cDNA was isolated.  $Ts^-$  growth and the altered length of telomeres in *cdc13-1* were restored by introduction of the *STM1* gene on a multi-copy vector, but an out-break of single-stranded telomeres in the *cdc13-1* cell was not restored. On the other hand, we found that a multi-copy of *SGS1*, encoding a helicase to unwind the guanine-quadruplex, inhibited suppression by *STM1* to *cdc13-1*. We found similarity in amino acid sequence between C-termini of Stm1 and  $\beta$ -subunit of telomere binding complex in *Oxytricha*. Telomere binding complex in *Oxytricha* consists of  $\alpha$  and  $\beta$ -subunits. The  $\alpha$ -subunit binds to single G-rich strand like as Cdc13, and the  $\beta$ -subunit binds to guanine quadruplex like as Stm1. We demonstrated that the fusion of N-terminal interaction region in Cdc13 and C-terminal region in Stm1, which had similarity to  $\beta$ -subunit, could complement the *CDC13* disruptant. Although *STM1* itself was not essential for telomere replication, our findings suggested that *STM1* genetically interacted with *CDC13* and functioned at the telomeres.

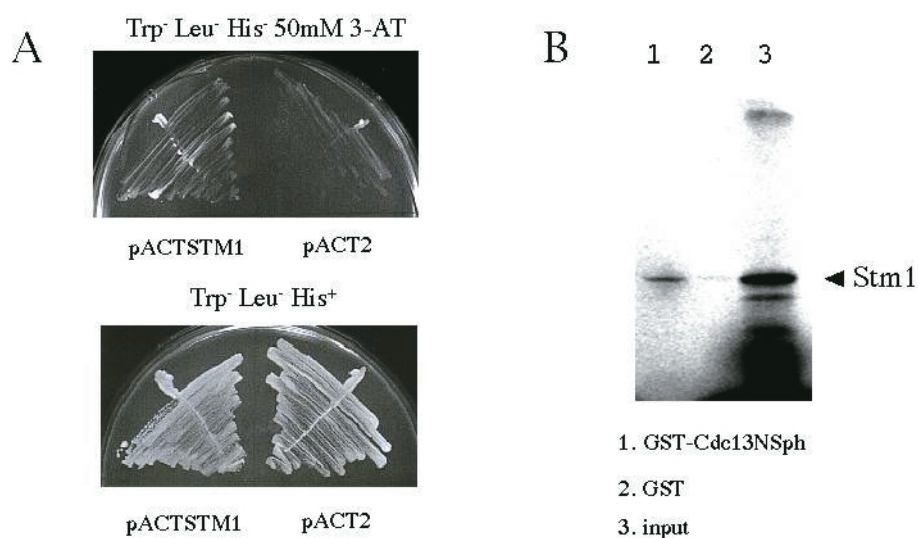


Figure. Stm1 binds to Cdc13. A. 2-hybrid analysis. Full length of *CDC13* was fused with GAL4 DNA binding region, and it was inserted into *CDC13* locus. Plasmid pACTSTM1 expresses fusion protein of Stm1 and GAL4 activation domain, and pACT is vectore alone. B. Pull-down experiment. In vitro synthesized Stm1 precipitated with N-terminal region of Cdc13 fused with glutatione S-transferase (GST).