The Highly Conserved tRNA$^{\text{His}}$ Guanylyltransferase Thg1p Interacts with the Origin Recognition Complex and Is Required for the G$_2$/M Phase Transition in the Yeast *Saccharomyces cerevisiae*

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Here we show that the *Saccharomyces cerevisiae* tRNA$^{\text{His}}$ guanylyltransferase Thg1p interacts with the origin recognition complex in vivo and in vitro and that overexpression of hemagglutinin-Thg1p selectively impedes growth of *orc2-ts* (Ts) cells at the permissive temperature. Studies with conditional mutants indicate that Thg1p couples nuclear division and migration to cell budding and cytokinesis in yeast.

In eukaryotes, DNA replication is linked to other cell cycle processes through the stepwise assembly of replication initiation complexes (1, 5, 13). Initiator function is provided by the origin recognition complex (ORC), which binds to replication origins and recruits factors required for assembly of prereplication complexes, a process known as origin licensing (3). ORC and many aspects of origin licensing are conserved in eukaryotes (7, 11). ORC also functions in heterochromatin-induced silencing of transcription in yeast (2, 6) and *Drosophila melanogaster* (8) and in chromosome condensation and segregation (16, 17).

ORC interacts with other factors, including the DNA bind-
ing proteins AIF-C1 and AIF-C2 (20), the transcription factor c-Myc (22), cyclin B/cdc2 complexes (24), and the HBO1 histone acetyltransferase (10). These and other interactions provide avenues for ORC to influence chromatin structure (10).

Here we show ORC interacts with Thg1p, the guanylyltransferase of *Saccharomyces cerevisiae* required for the addition of the G1 residue to the 5' end of histidine tRNA (9).

To identify proteins that interact with ORC, hemagglutinin (HA)-tagged Orc2p was fused to the DNA binding domain of Gal4p and used as bait in a two-hybrid screen as described previously (26). Y190 cells harboring the bait plasmid pAS1-ORC2 were transformed with an *S. cerevisiae* cDNA expression library; out of 2.6 × 10^6 transformants, 55 survived selection for histidine prototrophy and -galactosidase activity. After recovery in *Escherichia coli*, candidate plasmids were tested for specificity by mating to Y190 cells that contained[Y190 cells harboring the bait plasmid pAS1-ORC2 were transformed with an *S. cerevisiae* cDNA expression library; out of 2.6 × 10^6 transformants, 55 survived selection for histidine prototrophy and -galactosidase activity. After recovery in *Escherichia coli*, candidate plasmids were tested for specificity by mating to Y190 cells that contained]
either pAS1-ORC2 or unrelated bait plasmids. Eight of 55 plasmids encoded Thg1p (9), and β-galactosidase assays indicated that Thg1p interacts with Orc2p but not p53, Cdk2, PCNA, Cdc6, Snf1, or lamin (Fig. 1A). Gene disruption experiments showed that THG1 is essential for viability in S. cerevisiae (Fig. 1B), consistent with other reports (23). Incubation of 35S-methionine-labeled Orc2p with beads loaded with glutathione S-transferase (GST), GST-Y4 (Y4 control protein) or GST-scThg1p revealed weak but specific binding of Orc2p to GST-scThg1p (Fig. 1C, lane 7). This specific but weak interaction was also observed in coimmunoprecipitation assays (Fig. 1D, lane 4). Although tests for synthetic lethality between thg1(Ts) and orc2(Ts) strains were negative (data not shown), overexpression of HA-Thg1p markedly suppressed the growth of orc2(Ts) cells at the permissive temperature but not that of wild-type W303, orc5(Ts), cdc7(Ts), or cdc28(Ts) strains (Fig. 2A). The pattern of HA-Thg1p expression in orc2(Ts) cells also differed significantly from that in wild-type, orc5(Ts), or cdc7(Ts) strains (Fig. 2B). These differences were not dependent upon cell cycle arrest or cell death since they were not observed in orc5(Ts) or cdc7(Ts) strains at the restrictive temperature and were present in orc2(Ts) cells at both permissive and restrictive temperatures.

We next subjected THG1 to random mutagenesis (15) and isolated several temperature-sensitive alleles. When shifted to the nonpermissive temperature (37°C), cells containing the thg1-28(Ts) allele under the control of the endogenous THG1 promoter (19) showed a normal pattern of actin polarization to daughter buds during initial phases of bud formation at the restrictive temperature (data not shown). With time, however,
SYBR Green staining for DNA content (14) revealed that most cells collected in the G2/M phase of the cell cycle (Fig. 3B). After 3 h at the nonpermissive temperature many mutant cells displayed short thick bundles of tubulin (data not shown), suggesting that these cells were arrested in either prophase or G2 (12), where the intranuclear spindle is shorter than the metaphase spindle (21). mutant(HA) cells held at the semipermissive temperature of 33°C developed multibudded cells with markedly abnormal bud morphology (Fig. 3C). Staining for chitin with calcofluor (18) showed mother cells with as many as three to four daughter buds (Fig. 3D), indicating that new buds had emerged from daughter cells before cytokinesis had separated mother-daughter progenitor pairs.

Staining of mutant(HA) mutants with SYBR Green or 4,6-diamidino-2-phenylindole (DAPI) also revealed an unusual distribution of DNA in cells held at the nonpermissive temperature; in ~20% of multibudded cells mother cells completely lacked nuclear DNA, whereas daughter buds were positive for nuclear DNA (Fig. 3E to H). In many multibudded cells, the nucleus failed to divide but nonetheless was pulled into the daughter bud by an intact intranuclear spindle. This appears to have been followed by a new round of bud formation. Thus, the budding cycle that initiates at Start continues for multibudded cells at the nonpermissive temperature in mutant(HA) cells but is uncoupled from nuclear division and migration. Remarkably, mutant(HA) cells held at the restrictive temperature were viable, for multibudded mutant(HA) cells incubated for 16 h at 37°C in liquid culture formed colonies with the same efficiency as the control strain when plated and incubated at the permissive temperature (data not shown). mutant(HA) cells did not show increased sensitivity to UV light, the ribonucleotide reductase inhibitor hydroxyurea, or the thg1(Ts) cell held at the permissive temperature (data not shown).

Previously we showed that Clf1p, a factor required for pre-mRNA splicing, associates with replication origins in an ORC-dependent manner (26). Noc3p, a protein required for pre-rRNA processing, and Yph1p, a protein required for biogenesis of 60S ribosomal subunits, have also been reported to interact with ORC (4, 25). Here we link a factor involved in regulation of histidine tRNA biosynthesis to ORC, establishing an important connection between basic aspects of cell metabolism and cell cycle progression, perhaps in this instance through the nucleotide GTP. The Yph1p coding sequence is very highly conserved (9), and expression of the human protein rescues growth of yeast cells lacking THG1 as well as the yeast protein does (data not shown). We are presently investigating whether expression of TGH1 is required for progression through G2/M in human cells.

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