The WHI3 gene encodes an RNA binding protein that negatively regulates CLN3, a G1 cyclin that associates with Cdc28p to promote the G1/S phase transition. Whi3p regulates CLN3 both directly and indirectly. First, Whi3p contains a C-terminal RNA recognition motifthat binds CLN3 mRNA and localizes the mRNA into cytoplasmic foci, perhaps to locally restrict synthesis of this G1 cyclin. Second, the N-terminal Cdc28-recruitment region of Whi3p interacts with Cdc28p and G1 cyclin/Cdc28p complexes. In this capacity, Whi3p acts as a cytoplasmic retention factor, sequestering Cdc28p and associated cyclins in the cytoplasm of early G1 phase cells, thereby restricting the nuclear accumulation of these complexes to late G1 phase. Whi3p regulates the critical cell size required for passage through Start, the normal mating response, filamentous growth and meiosis.WHI3 was originally identified in a screen for small cell size mutants. Deletion mutants bud and enter S phase at a smaller volume than wild-type cells, suggesting a role for WHI3 in setting cell size at the G1/S phase transition. Transcriptional activation of G1/S phase genes including CLN2, CLB5 and SWI4 also occurs at a smaller cell size in the mutant than in wild-type cells. A comparison of the relative size of haploid and diploid strains containing 0-3 copies of the WHI3 gene indicate that WHI3 cell size modulation is dose-dependent. In keeping with these findings, overexpression of WHI3 produces a lethal G1 phase arrest where cells continue to increase in cell volume. The arrest requires the presence of an intact RRM and constitutive expression of CLN2 suppresses the arrest, although not the lethality. The effects of the whi3 mutation on cell size are additive with those of the hyperactive CLN3-1 allele, with double mutants smaller than either single mutant. Double mutants are unresponsive to alpha factor, including failure to arrest in G1, failure to induce alpha factor-responsive genes and failure to undergo the mating factor associated morphological transition. Although whi3 single mutants are fertile, whi3 CLN3-1 double mutants are nearly sterile, and this sterility can be reversed by deletion of CLN2. Although deletion of WHI4, a partially redundant homolog of WHI3, has no noticeable cell size defect on its own, it does exacerbate the small cell size phenotype of the WHI3 deletion, and results in slow growth. Overexpression of WHI4 also results in G1 phase cell cycle arrest and overexpression of either WHI3 or WHI4 represses the expression of mitotic cell cycle genes including G1 and S phase cyclins suggesting functional overlap.In addition to its effect on the mating response, Whi3p function is required for cells to undertake other developmental options such as filamentation and meiosis when challenged in G1 phase. Mutation of WHI3 blocks both pseudohyphal differentiation in diploids and invasive growth in haploids. Homozygous whi3 deletion strains are also defective for meiotic S phase entry and sporulation. The RNA recognition motif and Cdc28-recruitment region are essential for these functions, as mutants specifically deleted for either of these regions behave similar to the complete deletion. Deletion of CLN3 suppresses both the filamentous growth defect and the meiotic defect of the whi3 deletion strain.