Ribonucleotide reductaseis a tetrameric protein complex that catalyzes the conversion of nucleotides to deoxynucleotides, the rate-limiting step in de novo deoxyribonucleotide biosynthesis, and plays an essential role in DNA replication and repair. A balanced supply of deoxyribonucleoside triphosphatesis required for accurate genome duplication. Both the overall concentration and the balance among the individual dNTPsare tightly regulated by ribonucleotide reductase. Ribonucleotide reductase activity is periodic during the cell cycle, rising from an initial low level to a maximum early in S phase, then declining at the end of S phase. Ribonucleotide reductase consists of two large and two small subunits. In Saccharomyces cerevisiae, the main isoform of the large subunit is encoded by RNR1 and another isoform by RNR3; the two small subunits are encoded by RNR2 and RNR4. The Rnr1p:Rnr1p homodimer contains the regulatory and catalytic sites, and the Rnr2p:Rnr4p heterodimer houses the essential diferric-tyrosyl radical cofactor. The crucial role of Rnr4p is to fold correctly and stabilize the radical-storing Rnr2p by forming a stable 1:1 Rnr2p/Rnr4p complex. The contribution of RNR3 to ribonucleotide reduction is not clear. RNR3 is not expressed during normal growth, but like the other three subunits, is strongly induced by DNA damage, though never reaching more than one-tenth of the Rnr1p levels.During most of the cell cycle, Rnr1p and Rnr3p are localized to the cytoplasm, while Rnr2p and Rnr4p are present in the nucleus. In response to S phase or DNA damage, the Rnr2p:Rnr4p subcomplex undergoes checkpoint-dependent, nucleus-to-cytoplasm redistribution and binds the Rnr1p homodimer, forming an active RNR complex. Dif1p controls subcellular localization of the Rnr2p:Rnr4p subcomplex by binding directly to it and mediating its nuclear import. Wtm1p acts as a nuclear anchor to maintain nuclear localization of Rnr2p:Rnr4p outside of S phase or in the absence of DNA damage. Inhibition of ribonucleotide reductase activity by hydroxyurea treatment results in S phase cell-cycle arrest and large-budded, uninucleate cells. Both RNR1 and RNR2 are essential for viability, whereas RNR3 is not. Temperature-sensitive alleles of RNR1 and RNR2 arrest with a large-budded, cdc terminal phenotype at the nonpermissive temperature. Overexpression of RNR3 suppresses the lethality of rnr1 null mutations. Cells deleted for RNR3 are hypersensitive to rapamycin plus MMS. Deletion of RNR4 is lethal is some strain backgrounds but not in others, and this lethality can be suppressed by overexpression of RNR1 and RNR3, or of RNR2. Some rnr4 null mutants exhibit slow growth and sensitivity to mutagens, including UV light and psoralens, as well as increased sensitivity to oxidative stress. rnr4 null mutant cells are increased in size and also show higher budding frequency, pointing to a delay of mitosis/cytokinesis. RNR has been identified in E. coli, plantsand mammals. Because RNR activity is crucial for rapidly dividing cells, its overexpression can lead to neoplastic transformation, making RNR a target for cancer therapy. In mammalian cells, the RNR small subunit is the site of action of several antitumor agents, including hydroxyurea and 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone.