SIR2 encodes an NAD+-dependent deacetylase involved in chromatin silencing. Sir2p facilitates transcriptional silencing at cryptic mating type loci HML and HMR, at telomeres, and at the 0000183>rDNA locus RDN1, and this silencing regulates the processes of recombination, genomic stability, and aging. SIR2 is one of four Silent Information Regulator genes in yeast but is the only one that is highly conserved from archaea to humans. Although SIR2 is not essential for viability, absence of Sir2p function does result in a complete loss of transcriptional silencing, increases the rate of rDNA repeat recombination, decreases chromosome stability, causes defects in the meiotic pachytene checkpoint, and decreases yeast lifespan. Overexpression of Sir2p has been shown to extend the lifespan of yeast in a dose-dependant manner but highly overexpressed SIR2 can be toxic to the cells. Additionally, caloric restriction increases the activity of Sir2p while nicotinamide, a degradation product of NAD, inhibits it.Silencing at HML, HMR, and heterochromatic telomeres is mediated by the Sir complex, comprised of the two structural proteins Sir3p and Sir4p, as well as Sir2p which is the enzymatic component. The Sir complex does not bind DNA directly, instead it is recruited to regulatory chromosomal domains bound by Rap1p, Abf1p and the Origin Recognition Complex. Once a silencing complex is bound to a nucleosome, Sir2p deacetylates the histone tails of H3 and H4 of the adjacent nucleosome. Because the Sir proteins have a higher affinity for H3 and H4 with reduced acetylation, deacetylation creates a binding site for an additional silencing complex. This process repeats until Sir complexes are spread across the entire chromatin region to be silenced.Sir2p also localizes to the nucleolus. Sir2palong with Net1p and the Cdc14p phosphatase comprise the nucleolar complex called RENT, a regulator of nucleolar silencing and telophase exit. The association of Sir2p to rDNA is dependent on Net1p, and RENT is recruited to rDNA through interaction with Fob1p and RNA polymerase I. As a component of RENT, Sir2p represses mitotic and meiotic recombination between rDNA arrays, and affects rDNA chromatin structure and silencing in a dose-dependent manner. Sir2p may play a role in slowing the aging of yeast cells by preventing the formation of extrachromosomal rDNA circlesthat form through homologous recombination within rDNA arrays, and that seem to be one cause of yeast cell agingAbout NAD biosynthesis -- de novo and salvage pathways Nicotinamide adenine dinucleotideis an essential cofactor for cellular redox reactions and energy metabolism. NAD also has been shown to be an important substrate in a variety of biological processes, including transcriptional regulation, DNA repair, calcium-dependent signaling pathways, calorie-restriction-mediated life-span extension and age-associated diseases. NAD appears to affect these processes by regulating the Sir2p family of NAD-dependent deacetylases. There are a number of pathways for NAD biosynthesis. In yeast and most other organisms, the two major pathways are de novo synthesis of NADand regeneration of NAD from its nicotinamide degradation products. NAD is synthesized de novo from tryptophan via kynurenine. In this pathway tryptophan is converted to nicotinic acid mononucleotidein 6 enzymatic stepsand one non-enzymatic step. At NaMN the de novo pathway converges with the NAD salvage pathway and the last two steps to NAD are shared. In the yeast NAD salvage pathway, the vitamin precursors nicotinamide and nicotinic acid are converted to NaMN, the point of convergence with the de novo pathway. The steps from nicotinic acid to NAD were elucidated by Preiss and Handler and are sometimes referred to as the Preiss-Handler pathway. Yeast can also import extracellular nicotinic acid into the cell by the permease Tna1p and then convert it to NAD via the Preiss-Handler pathway. There are four additional pathways for synthesizing NAD in yeast: two salvage pathways from the vitamin precursor nicotinamide ribosideand two salvage pathways from nicotinic acid riboside. Only one of these pathways, the NR salvage pathway I, is independent of the NAD salvage pathway. In the NR salvage pathway I, NR is phosphorylated to nicotinamide mononucleotide by the kinase Nrk1p, and then adenylated to NAD by Nma1p or Nma2p. In the NR salvage pathway II, the hydrolase Urh1p or the phosphorylase Pnp1p split NR into a ribosyl product and nicotinamide, which subsequently is converted to NAD via the NAD salvage pathway. The initial steps in the NaR salvage pathways I and II are similar to those of the NR salvage pathways I and II and are catalyzed by the same enzymes, respectively. In the NaR salvage pathway I, Nrk1p phosphorylates NaR to NaMN, which subsequently is converted to NAD via the enzymes shared by the de novo and NAD salvage pathways. In the NaR salvage pathway II, Urh1p or Pnp1p split NR into a ribosyl product and nicotinic acid, which is first converted to NaMN and then is converted similarly to NAD.