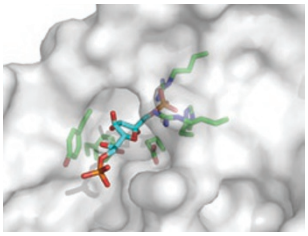
Ribose production on demand



Eukaryotic cells consume glucose through glycolysis and the oxidative pentose phosphate pathway, which produces NADPH and the essential nucleotide component ribose-5-phosphate. Glycolytic intermediates can also be converted into ribose by enzymes in the nonoxidative arm of the pathway, the regulation of which is poorly understood. By carrying out a metabolomics screen of yeast deletion mutations of genes of unknown function, Clasquin *et al*. found that deletion of *SHB17* causes the accumulation and depletion of certain metabolites. Biochemical assays helped determine the endogenous substrates of Shb17, sedoheptulose-1,7-bisphosphate (SBP) and octulose-1,8-bisphosphate (OBP), which are converted into sedoheptulose-7-phosphate (S7P) and octulose-8-phosphate (O8P), respectively. Shb17 had previously been shown to have phosphatase activity against the structurally similar metabolite fructose-1,6-bisphosphate (FBP) *in vitro*, but FBP does not accumulate in the *SHB17* deletion strain, and kinetic studies confirmed Shb17's preference for SBP. Structural analysis of the Shb17–SBP complex revealed strong similarities to the recently determined structure of Shb17 in complex with FBP. However, Shb17 makes additional hydrogen bond interactions with SBP, and SBP binds the active site in a more favorable closed furan conformation, which may explain its higher affinity. Isotope labeling studies allowed the quantification of carbon-to-ribose flux: flux through Shb17 increases when ribose demand is high relative to the demand for NADPH. In metabolically synchronized yeast cells, Shb17 expression levels are correlated with expression levels of ribosomal proteins, suggesting that periodic Shb17 expression coincides with the peak demand for ribose phosphate that occurs during ribosome synthesis. Together, these findings suggest that Shb17 links the pentose phosphate pathway and glycolysis in a sequence of reactions—called riboneogenesis—by catalyzing a strongly thermodynamically driven dephosphorylation step. Through this process, the riboneogenesis pathway converts glycolytic intermediates into ribose-5-phosphate without the production of NADPH, allowing the cell to adjust the flux of carbon to ribose in response to changing conditions. (*Cell* **145**, 969–980, 2011) *AH*

Heating up the nuclear pore

The nuclear pore complex (NPC) is a massive cylindrical structure that bridges the inner and outer membranes of the nuclear envelope and acts as a gatekeeper, preventing the passive diffusion of large biomolecules into and out of the eukaryotic cell nucleus. This ~50-MDa, highly symmetric complex is comprised of approximately 30 different nucleoporins, and the enormity of the NPC has meant that there is limited information about the overall structure and relative orientations of the various components. Amlacher *et al.* previously used a yeast two-hybrid analysis to determine nucleoporin-nucleoporin interactions, but they found that many of the yeast nucleoporins were not stable *in vitro*, which prohibited the authors from conducting follow-up biochemical and structural studies. For this reason, they turned to *Chaetomium thermophilum*, a thermophilic fungus whose recombinant nucleoporins were well behaved *in vitro*. The authors first used recombinant nucleoporins from this thermophile to confirm, *in vitro*, the protein-protein interactions seen by yeast two-hybrid analysis. They then used electron microscopy to examine the structures of individual nucleoporins and various co-complexes. These analyses revealed that Nup192 and Nup188 have similar S-shaped structures, despite their limited sequence homology; the shape and curvature of half of the 'S' shape is reminiscent of the structures of karyopherin transport receptors such as exportin-t and Crm1. This raises the intriguing possibility that those transport receptors evolved from an ancestral nucleoporin, perhaps one that lost its ability to assemble into the NPC and also gained a nuclear export sequence motif. By integrating the information from their biochemical and structural studies, the authors were able to propose a model for the inner pore ring complex of the NPC. In this model, Nup192 and Nup170, which are fairly large, are linked together through interactions with short, flexible regions of Nup53 and Nic96. Because 73% of the 5,797 *Saccharomyces cerevisiae* proteins and 44% of the 22,937 human proteins have homologs in *C. thermophilum*, the authors propose that this thermophilic fungus will be useful for structural studies of other eukaryotic protein complexes, especially when the yeast or human variants are unstable or poorly behaved *in vitro*. (*Cell* **146**, 277–289, 2011) *JMF*

Border protection

Meiotic recombination is the hallmark of sexual reproduction, allowing parental genes to be mixed and shuffled for the next generation. However, homologous recombination events might be too dangerous in some regions of the genome. For instance, repetitive DNA sequences are prone to nonallelic homologous recombination. These events can result in copy number variations in the progeny as well as catastrophic genomic rearrangements that are associated with diseases and birth defects. To prevent such events, budding yeast *Saccharomyces cerevisiae* blocks the formation of meiotic double-strand breaks (DSBs) within the repetitive ribosomal DNA (rDNA) array, partly through formation of heterochromatin in a Sir2-dependent manner. The meiosis-specific AAA-ATPase Pch2 was also known to suppress recombination at the rDNA site, though its exact role was unclear. Now, Hochwagen and colleagues examine these issues in detail and find that the borders of the rDNA array are actually highly susceptible to meiotic DSBs and that yeast cells have a mechanism to specifically protect those regions. This border protection system uses Pch2 and the protein Orc1, a subunit of the origin recognition complex involved in initiation of DNA replication. Deficiencies of Pch2 or Orc1 led to higher rates of DSBs and nonallelic recombination at the rDNA borders, resulting in changes in the number of rDNA repeats in the progeny. Moreover, chromatin immunoprecipitation (ChIP) analyses revealed that Pch2 blocks the recruitment of DSB factors Rec114, Mer2 and Mre11 to the rDNA borders. The effects of the *pch2* mutation could be largely suppressed by mutation of histone deacetylase Sir2, indicating that though Sir2 protects the bulk of the rDNA from meiotic DSBs, it induces their formation at the junction of heterochromatic and euchromatic regions. The reasons for this remain unknown, and, though heterochromatin marks vary between organisms, it will be interesting to see whether similar mechanisms operate in higher eukaryotes. (*Nature* doi:10.1038/nature10331, published online 7 August 2011) *IC*

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