Quantification of protein content - dehydrogenases

Table S7-1 Description of reactions catalyzed by the proteins under consideration

Gene name /protein	Description
IDH1/YNL037C	Subunit of mitochondrial NAD-dependent isocitrate dehydrogenase; Complex catalyzes the oxidation of isocitrate to alpha-ketoglutarate in the TCA cycle
IDH2/YOR136W	Subunit of mitochondrial NAD-dependent isocitrate dehydrogenase; Complex catalyzes the oxidation of isocitrate to alpha-ketoglutarate in the TCA cycle; phosphorylated
IDP1/YDL066W	Mitochondrial NADP-specific isocitrate dehydrogenase
IDP2/YLR174W	Cytosolic NADP-specific isocitrate dehydrogenase; catalyzes oxidation of isocitrate to alpha-ketoglutarate; levels are elevated during growth on non-fermentable carbon sources and reduced during growth on glucose
ALD4/YOR374W	Mitochondrial aldehyde dehydrogenase; required for growth on ethanol and conversion of acetaldehyde to acetate; phosphorylated; activity is K+ dependent; utilizes NADP+ or NAD+ equally as coenzymes; expression is glucose repressed; can substitute for cytosolic NADP-dependent aldehyde dehydrogenase when directed to the cytosol
ALD5/YER073W	Mitochondrial NADP-Acetaldehyde dehydrogenase, Mitochondrial aldehyde dehydrogenase; involved in regulation or biosynthesis of electron transport chain components and acetate formation; activated by K+; utilizes NADP+ as the preferred coenzyme; constitutively expressed
ALD6/YPL061W	Cytosolic aldehyde dehydrogenase; activated by Mg2+ and utilizes NADP+ as the preferred coenzyme; required for conversion of acetaldehyde to acetate; constitutively expressed; locates to the mitochondrial outer surface upon oxidative stress
GDH1/YOR375C	NADP(+)-dependent glutamate dehydrogenase; synthesizes glutamate from ammonia and alpha-ketoglutarate; rate of alpha-ketoglutarate utilization differs from Gdh3p; expression regulated by nitrogen and carbon sources; GDH1 has a paralog, GDH3, that arose from the whole genome duplication
GDH2/YDL215C	NAD(+)-dependent glutamate dehydrogenase; NAD(+)-dependent glutamate dehydrogenase; degrades glutamate to ammonia and alpha-ketoglutarate; expression sensitive to nitrogen catabolite repression and intracellular ammonia levels
GDH3/YAL062W	NADP-dependent glutamate dehydrogenase
ZWF1/YNL241C	Glucose-6-phosphate dehydrogenase (first step in PPP). Regenerates NADPH
GND1/YHR183W	6-phosphogluconate dehydrogenase (decarboxylating); catalyzes an NADPH regenerating reaction in the pentose phosphate pathway

It was found that the cytosolic NADP-dependent isocitrate dehydrogenase (IDP2p) showed a relative lower content at D=0.3 h^{-1}, while the mitochondrial NAD-dependent IDH1p and IDH2p seemed to increase with the growth rate. IDH2p was the protein that showed the largest relative change with the growth rate. The mitochondrial IDP1p also showed an increasing trend with the growth rate.

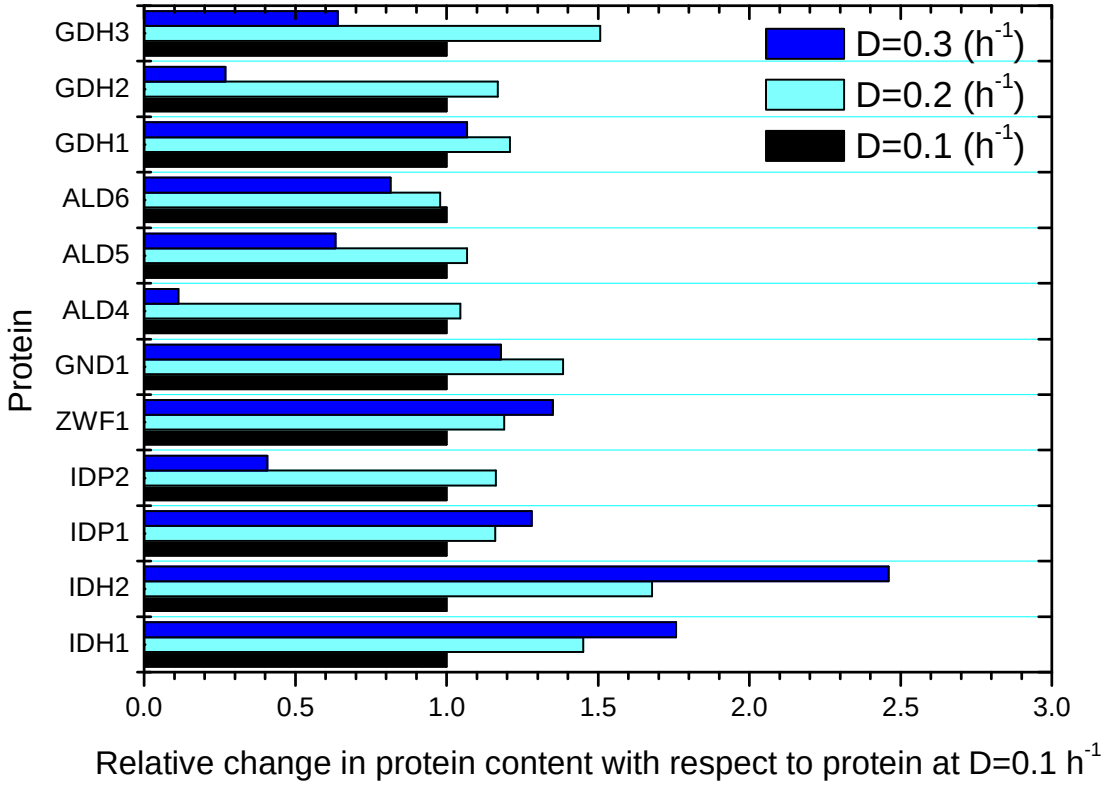

Figure S7-1 Relative changes in protein content of hydrogenases. The measured ratios were normalized to the value corresponding to growth rate D=0.1 h^{-1}

Protocol

Selected reaction monitoring (SRM) was used to determine the proteins relative to a 15N-labeled yeast cell lysate that was used for normalization of the various measurements. The general SRM workflow has been described previously (references 1, 2 below) and was used with the following minor adjustments/variations to target proteins listed in Table S7-1:

- The 15N standard was created from a mix of yeast cultures in the mid-exponential growth phase, cell in the diauxic shift and cells in the stationary phase, rather than cells only in the diauxic shift phase
- Protein extraction was performed using lysis with sodium hydroxide rather than using glass beads
- Samples were measured on a triple quadrupole MS (TSQ Vantage, Thermo) coupled to nano-LC system (Dionex) containing a reverse phase C18 column

Data processing

- Data was manually curated using the Skyline software (MacLean, B (2010), Bioinformatics, 26(7),966-8)
- The ratio of each peptide was calculated relative to the isotopically labeled standard via integration of the peak areas (and the average was taken for multiple peptides to calculate the relative protein concentration)

- Global data correction was performed by normalization to the median (median based on >700 peptides targeting >200 proteins. The data presented is a subset of this dataset) to correct for global variations in the protein concentrations
- Relative changes in protein concentrations were obtained via comparison of the protein concentration in one sample to the other.

References

1. Picotti, P., Bodenmiller, B., Mueller, L.N., Domon, B., and Aebersold, R. (2009) Full dynamic range proteome analysis of S. cerevisiae by targeted proteomics. Cell. 138, 795-806

2. Costenoble, R., Picotti, P., Reiter, L., Stallmach, R., Heinemann, M., Sauer, U., and Aebersold, R. (2011) Comprehensive quantitative analysis of central carbon and amino-acid metabolism in Saccharomyces cerevisiae under multiple conditions by targeted proteomics. Mol.Syst.Biol. 7, 464