Regulation of the glycerol pathway during osmotic stress

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Adaptation of cell to a perturbation can occur by adjusting gene expression and metabolic changes. How the cell actually adapts will depend on the environment, the history of the cell and the type of perturbation. Adaptation to osmotic stress in yeast involves the induction of glycerol production and closure of the glycerol channel. The increase of glycerol production is perceived to be caused by an increase in the expression of glycerol producing enzymes. However, post-translational modification of enzymes in the glycerol pathway or metabolic changes upon an osmotic shock may also lead to an increase in glycerol production.

We quantified to what extent the increase in glycerol production upon an osmotic shock is caused by changes in transcription, translation and/or metabolism. We perturbed a glucose-limited chemostat with a sustained shock of 1 M of sorbitol and analyzed the RNA levels, protein levels, enzyme activities of the glycerol producing enzymes and the glycerol flux in time. RNA levels were analyzed using quantitative real-time PCR and protein levels for GPD1 by western blot. The transcript levels of GPD1, GPP1 and GPP2 and GPD1 protein levels were shown to be increased after an osmotic shock, as was previously shown for batch cultures. We analyzed the V_{max} of glycerol-3-dehydrogenase and glycerol-phosphatase in an offline assay. The enzyme activity of both enzymes increased upon the shock. Total glycerol levels in the chemostat were monitored by an offline enzymatic determination. From these values the glycerol flux was calculated. The glycerol flux first increased then remained constant for 10 min before increasing again.

Regulation analysis (Rossell et al., 2005) can show to what extend a change in flux is caused by a change in gene expression and to extend it is caused by metabolic changes.

The flux through an enzyme can be described by this equation:

$$v = V\max \cdot \left[F(S, P, Ks, Kp, \ldots.) \right]$$

As the V_{max} depends on the amount of enzyme, gene expression can alter V_{max}. The second part of the enzyme rate equation describes the metabolic effects on an enzyme.

In logarithmic space the multiplication becomes a summation and the relative change from one condition to another can be depicted by delta:

$$\Delta \ln(v) = \Delta \ln(V\max) + \Delta \ln[F(k_{cat}, S, P, Ks, Kp, ...)]$$

Dividing all terms by $\Delta \ln(v)$ gives you:

$$1 = \frac{\Delta \ln(V\max)}{\Delta \ln(v)} + \frac{\Delta \ln[F(k_{cat}, S, P, Ks, Kp, ...)]}{\Delta \ln(v)}$$

These terms are renamed to:

$$1 = \rho_{hierarchical} + \rho_{metabolic}$$

The V_{max} is more easily measured then the metabolic effects, therefore ρh is readily calculated. The summation theorem makes it possible to deduce the metabolic effect from this.

$$\rho_h = \frac{\Delta \ln V\max}{\Delta \ln(v)} \quad \text{and} \quad \rho_{metabolic} = 1 - \rho_{hierarchical}$$

In our case we studied the change in glycerol flux relatively to the steady state condition and we calculated the hierarchical coefficient:

$$\rho_h = \frac{\ln V\max_t - \ln V\max_{ss}}{\ln v_t - \ln v_{ss}}$$

Analysis with this method shows that the flux through the glycerol pathway is first only regulated at the metabolic level and after several minutes is only partly regulated at the hierarchical (gene expression) level.

We hypothesize that the shrinkage of the cell leads to metabolic changes that increases the activity of glycerol producing enzymes that were already present. Currently we are studying what metabolic effect may cause the increase in glycerol flux.

References

- Rossell S, van der Weijden CC, Kruckeberg AL, Bakker BM, Westerhoff HV (2005) FEMS Yeast Res. 5:611-9.