

Cell economics; ATP as a potential metabolic messenger

Student project – Molecular Systems Biology

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Introduction

In response to the amount and type of nutrients available, cell metabolism is highly adaptable in the yeast *Saccharomyces cerevisiae*. In a high glucose environment cells will ferment and produce large quantities of ethanol, which will be respired by the same cells after glucose depletion. The amount of energy produced by a cell per time unit can vary tremendously, Cells respond to these changes by adapting their behavior, e.g. they reduce their growth rate and build up carbon storage.

The major energy currency of the cell is adenosine triphosphate (ATP). The high energy potential of ATP is crucial for many biological processes, such as the synthesis, transport and degradation of biological molecules. In addition, ATP may play an important role in controlling the activity of metabolic pathways (Szewczyk & Pikuła, 1998; Larsson *et al.* 2000), e.g. by variations in its intracellular concentrations.

Recently it became possible to measure intracellular ATP levels in real time using a fluorescence resonance energy transfer (FRET) sensor (Imamura *et al.* 2009; Bermejo *et al.* 2011). Upon exposure to e.g. laser light, fluorescent proteins can become excited. The acquired energy can be emitted as light, but the energy can also be transferred to another fluorescent protein. This latter event is referred to as FRET. The distance between the fluorescent proteins determines how efficient the energy transfer is.

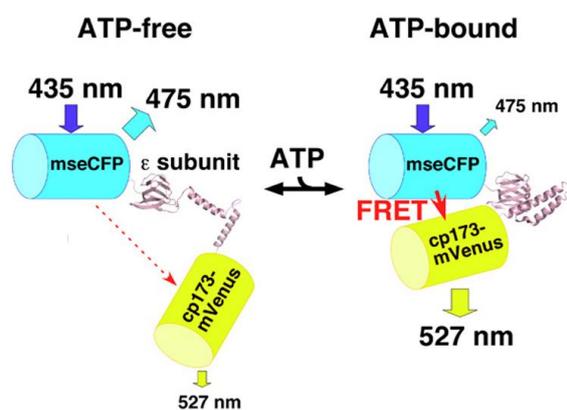


Figure 1: Schematic drawing of the ATP FRET sensor (Imamura *et al.* 2009). Upon excitation of mseCFP (dark blue arrow), the acquired energy can be emitted as light (light blue arrow) or transferred to mVenus (red arrow). The two proteins are connected together by an ATP binding protein (ϵ subunit of an ATP dehydrogenase). dependent on whether ATP is bound or not, the fluorescent proteins

The ATP FRET sensor contains three different parts: two fluorescent proteins (mseCFP and mVenus) and an ATP binding protein (Figure 1). When ATP binds to the sensor, it causes a conformational change and the two fluorescent proteins move closer together. Energy can now be transferred with an increased efficiency. By comparing the fluorescence intensities of mseCFP and mVenus, changes in FRET efficiency and thus ATP concentration can be measured.

Goal

We would like to measure ATP levels in a yeast cell during respiration, fermentation and metabolic shifts between these modes.

What to expect

→ Basic lab techniques, such as culturing yeast and simple genetic work, such as introduction of the ATP FRET sensor

→ A lot of fluorescence microscopy in combination with microfluidics (see also Lee *et al.* 2012)

→ Analysis of microscopy data using programs such as ImageJ/CellProfiler

The pictures made during the experiments will be analysed using image analysis software, like ImageJ, and/or Matlab to determine the intracellular ATP levels.

Literature:

Bermejo *et al.* (2011) In vivo biochemistry: quantifying ion and metabolite levels in individual cells or cultures of yeast. *Biochem. J* **438** (1–10).

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Lee *et al.* (2012). Whole lifespan microscopic observation of budding yeast aging through a microfluidic dissection platform. *PNAS* **109**: 4916-4920.

Szewczyk & Piłkuła (1998) Adenosine 5'-triphosphate: an intracellular metabolic messenger. *Biochim Biophys Acta* **1365**: 333-353.