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Study on Oxygen Sensing in Yeast

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Most organisms have respiratory system for energy generation, and use oxygen as electron acceptor for respiration. However, on being shifted from an aerobic to an oxygen-depleted environment, many processes within the cell have to adapt to the new conditions. For example, in the yeast *Saccharomyces cerevisiae*, energy generation exchanges fermentation from respiratory on an anaerobic condition.

Moreover, the place, where oxygen-requiring reactions take place, is not only the respiratory chain, but also other organelle, *e.g.*; peroxisome, on which β-oxidation and other oxidative pathways are located. Therefore, organisms must recognize oxygen concentrations on growth condition, and must maintain the active balance between respiration and other metabolism using oxygen, making adaptation to the concomitantly changing environmental conditions possible.

On the other hand, the methylotrophic yeast, which is able to utilize methanol as a sole carbon source, proliferated large peroxisomes, in which alcohol oxidase (AOD), key enzyme on methanol metabolism, is located. In methanol metabolism, oxygen is consumed by AOD on peroxisome, as well as by respiratory on mitochondrion. Therefore, it seems that methanol-metabolic pathway is able to use as model pathway for examining the molecular mechanism of oxygen sensing. In this study, we investigated regulation of AOD-gene expression by oxygen in methylotrophic yeast *Pichia methanolica*.

1. Expresstion of AOD isozyme by oxygen

We had reported that *P. methanolica* has nine AOD isozyme, which is octamer consisted of two subunits encoded by two AOD genes, *MOD1* and *MOD2*, and the band profile of an AOD zymogram reflects the composition ratio of two subunit species (1). At first, we observed effect of oxygen concentrations on induction of AOD isozyme. The AOD isozyme was not induced under anaerobic conditions, although showed nine forms on aerobic condition. Moreover, the composition of the AOD-isozyme species responded to the oxygen concentration. With an increase in the oxygen concentration, the ratio of Mod2p to Mod1p increased (Fig. 1A). These zymogram patterns and the gene-expression patterns of *MOD1* and *MOD2* coincided each other. With an increase in the oxygen concentration, the *MOD1* mRNA level decreased and the level of *MOD2* expression was becoming dominant. Therefore, the regulation of AOD isozyme by oxygen was confirmed to be controlled mainly at the mRNA level.

2. Regulation of function balance between AOD isozyme and respiratory

It seems that the balance of oxygen consumption between AOD activity and respiratory function is important for methanol metabolism. Next, we added the respiratory inhibitor, *i.e.*, antimycin A and sodium azide, to AOD-inducible condition, in order to observe effect of respiratory function on induction of AOD isozyme. Presence of respiratory inhibitors repressed induction of AOD isozyme. With an increase in inhibitor concentration the level of Mod1p was becoming dominant, although Mod2p was dominant on low concentration of respiratory inhibitor. Moreover, zymogram pattern by addition of inhibitor is very similar to that showed by the change of oxygen concentrations (Fig. 1B). These results indicate that respiratory

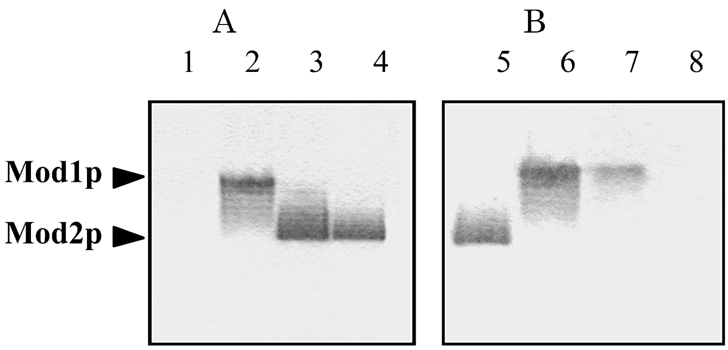

Fig. 1. Zymogram patterns of AOD-isozyme complexes from the *P. methanolica* induced with (A) various concentrations of oxygen, and (B) various concentrations of sodium azide. Electrophoresis was carried out on a 5% polyacrylamide gel. The oxygen concentrations were controlled by shaking speed: lane 1, anaerobic condition; lane 2, 50 rpm; lane 3, 100 rpm; and lane 4, 150 rpm. The sodium azide concentrations were; lane 5, 0%; lane 6, 1×10^{-3}%; lane 7, 2×10^{-3}%; and lane 8, 3×10^{-3}%.

function is essential for the induction of AOD isozyme.

3. Oxygen-metabolic balance and crosstalk between organelles

In this study, we showed that oxygen sensing in AOD-gene expression involves respiratory function, and it seems that mitochondrion releases some signals that function in the AOD-gene expression. That is to say, AOD-gene expression is controlled according as respiratory function, and there are crosstalks for oxygen sensing between some organelles, *e.g.*; mitochondrion-nuclear-peroxisome, to maintain oxygen-metabolic balance in the cell.

1) Nakagawa T, Mukaiyama H, Yurimoto H, Sakai Y, Kato N. 1999. Alcohol oxidase hybrid oligomers formed *in vivo* and *in vitro*. Yeast **15**: 1223–1230.