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Small-scale hypoxic cultures for monitoring the spatial reorganization of glycolytic enzymes in *Saccharomyces cerevisiae*

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Abstract:

At normal oxygen concentration, glycolytic enzymes are scattered in the cytoplasm of *Saccharomyces cerevisiae*. Under hypoxia, however, most of these enzymes, including enolase, pyruvate kinase, and phosphoglycerate mutase, spatially reorganize to form cytoplasmic foci. We tested various small-scale hypoxic culture systems and showed that enolase foci formation occurs in all the systems tested, including in liquid and on solid media. Notably, a mall-scale hypoxic culture in a bench-top multi-gas incubator enabled the regulation of oxygen concentration in the media and faster foci formation. Here, we demonstrate that the foci formation of enolase starts within few hours after changing the oxygen concentration to 1 % in a small-scale cultivation system. The order of foci formation by each enzyme is tightly regulated, and of the three enzymes, enolase was the fastest to respond to hypoxia. We further tested the use of the small-scale cultivation method to screen reagents that can control the spatial reorganization of enzymes under hypoxia. An AMPK inhibitor, dorsomorphin, was found to delay formation of the foci in all three glycolytic enzymes tested. These methods and results provide efficient ways to investigate the spatial reorganization of proteins under hypoxia to form a multienzyme assembly, the META body, thereby contributing to understanding and utilizing natural systems to control cellular metabolism via the spatial reorganization of enzymes.

Keywords: glycolytic enzymes, META body, *Saccharomyces cerevisiae*, small-scale hypoxic culture, spatial reorganization, time-scale monitoring