without an intervening endothelial barrier (Fig. 4), or, more commonly, erythrocyte formation took place on the edge of the explant and red cells were released to float on the fluid film surrounding the tissue (Fig. 5). Unless some endoderm was present, formation of a compact blood island and endothelium did not occur. Recombination at the time of explantation of the ectomesoderm with endoderm resulted in formation of typical blood islands and normal differentiation.

It is concluded that hemoglobin synthesis and blood island formation are dissociable events. While the mesoderm can carry out erythropoiesis in the absence of endoderm, the endoderm influences the frequency of erythrocyte formation and orients the mesoderm into blood islands in which endothelium formation can take place. Mere condensation into a blood island, however, is not a sufficient condition for erythropoiesis, for derangement of nucleic acid metabolism prior to the head-process stage allows formation of blood islands (*7*) without subsequent synthesis of hemoglobin.

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Tricarboxylic Acid Cycle Mutants in Saccharomyces: Comparison of Independently Derived Mutants

Abstract. *A yeast mutant independently isolated as a glutamate auxotroph* (glt_{2-1}) *was similar to the* glt_{1-1} *mutant in exhibiting a blocked tricarboxylic acid cycle due to the lack of aconitate hydratase. The new mutant differed by exhibiting blocks in lysine and cytochrome biosynthesis which segregated together with the glutamate requirement.*

We recently described (*1*) some of the characteristics of a glutamate-requiring yeast mutant (glt_{1-1}) which lacked aconitate hydratase. A second glutamate auxotroph (glt_{2-1}) independently induced by ultraviolet irradiation (*2*) was nonallelic to glt_{1-1}. When the two mutant strains were mated, glutamate independent hybrids were always obtained. Tetrad analysis of the four-spored asci obtained from these diploid hybrids showed a high frequency of prototrophic recombinants and the independent assortment of glt_1 and glt_2 markers predicted for unlinked genes.

The glutamate requirement in both mutant strains is due to the lack of aconitate hydratase, and this lack leads to the failure of the mutants to make α-ketoglutarate (the immediate precursor of glutamate) by way of the tricarboxylic acid cycle. It thus appears that at least two unlinked genes take part in the biosynthesis of aconitate hydratase. The possibility that two polypeptides, one controlled by each gene, might participate in aconitate hydratase structure or activity was tested by assaying mixed, cell-free preparations derived from each mutant under a variety of preparative and assay conditions. Complementation in vitro was not observed but this may still be a matter of technical difficulty since the mutants complement each other in vivo. Aconitate hydratase activity per milligram of protein (*3*) in the complemented diploid (*362 ± 13 units*) was comparable to that in a wild-type diploid (*323 ± 77 units*).

In addition to the glutamate requirement based on a lack of aconitate hydratase, glt_2 mutant strains required lysine for growth and lacked cytochromes *a* and *b*. This complex phenotype segregated regularly as a unit; glutamate-requiring segregants required lysine and were nonrespiring with all substrates tested. Regular segregation of the inability to respire has been seen before in the "segregational petites" (*4, 5*). The current phenotype is similar to that of the ly_6 and ly_8 mutants in which a requirement for lysine and a block in cytochrome biosynthesis segregated as a unit and appeared in each case to be primarily due to an alteration in a single gene (*5*). We, therefore, examined the ly_6 and ly_8 mutants for a glutamate requirement, which had, up to this point, not been reported. Indeed they do require glutamate for growth. This requirement was also based on the lack of aconitate hydratase. These mutants are thus all of similar phenotype even though the glt_2 mutant was isolated as a glutamate auxotroph and the ly_6 and ly_8 mutants as lysine auxotrophs. By contrast no requirement for lysine is associated with the glt_1 marker and no requirement for glutamate with the ly_1, ly_2, ly_3, ly_4, ly_5, ly_7, ly_9, or ly_{10} markers.

Complementation tests on media lacking lysine indicate that the glt_2 lesion is probably heteroallelic to ly_8 and that ly_6 is nonallelic to ly_8 and glt_2.

Complementation tests were also performed against appropriate petite test strains. The results indicated that most glt_2 segregants were ρ^- (lack the cytoplasmic factor for cytochrome biosynthesis). From 5 to 10 percent of the strains, which contained 0.01 to 0.03 percent of ρ^+ cells, exhibited a very weak ρ^+ response.

One interesting problem raised by the isolation of these mutants is the interrelation between and control of glutamate, lysine, and cytochrome biosynthesis. α-Ketoglutarate is a common precursor in all three pathways. The block in the biosynthesis of aconitate hydratase does not appear to interfere with lysine and cytochrome biosynthesis in the glt_1 mutant but does appear to in the glt_2, ly_6, and ly_8 mutants. The mechanism underlying this difference is not yet known.

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