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GENETIC MANIPULATION OF A LATENT DEFECT IN YEAST CYTOCHROME BIOSYNTHESIS UTILIZING CYTODUCTION*

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SUMMARY. The effects of a *mit*$^-$ mutation, *oxi2*, and the ρ° mutation on expression of a defective nuclear structural gene for δ-aminolevulinic acid synthase (*cyd1*) were compared. The technique of cytoduction was used to introduce *oxi2* mitochondria into a *cyd1*ρ° recipient cell, thereby permitting comparison of isonuclear strains. Like ρ°, the *oxi2* mutation caused an apparent unmasking of the cytochrome deficiency associated with the *cyd1* mutation, provided cells were grown on glucose. When *cyd1* strains with ρ$^+$, ρ° or *oxi2* mitochondrial genotypes were grown on galactose medium, substantial cytochrome formation occurred in each case. It is concluded that the exacerbation of the *cyd1*-dependent cytochrome deficiency by ρ° or *oxi2* mutations depends upon glucose repression. However, derepression resulting from growth on galactose medium does not fully overcome the *cyd1* defect, since both *cyd1*ρ° *oxi2* strains require added δ-aminolevulinic acid for maximum cytochrome biosynthesis.

Evidence that mitochondria regulate heme biosynthesis at the level of δ-aminolevulinic acid (Alv) formation was provided by Sanders et al. (1) who demonstrated a synergistic effect of the ρ° mutation on the phenotypic expression of a mutant nuclear gene, *cyd1*, which probably controls the primary structure of Alv synthase (2,3). The *cyd1* defect is largely latent in normal ρ$^+$ strains, but when mitochondrial DNA function is destroyed by mutation to the ρ$^-$ or ρ° state, the resulting cells produce extremely low levels of cytochrome *c*. Normal cytochrome *c* synthesis can be restored to *cyd1*ρ° cells by adding Alv to growth medium (1). The object of the present investigation was to determine

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Abbreviation used: Alv - δ-aminolevulinic acid.

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whether the unmasking of the latent defect by the ρ° mutation could be attributed merely to loss of respiratory function or whether some specific mitochondrial gene residing in normal mitochondrial DNA is necessary to maintain the latent state. To this end the technique of cytoduction (4) was employed to construct a cytochrome oxidase-deficient strain containing a *cyd1* mutation in the nucleus and an *oxi2* mutation in the mitochondrial genome. Cytoduction utilizes the nuclear-fusion mutation *kar1* which permits transfer of a mitochondrial genome from one haploid strain to another without altering either nucleus.

MATERIALS AND METHODS

Yeast strains

The genotypes and sources of the strains used in this study are given in Table 1. Cytoduction of mitochondrial genomes to ρ° acceptor cells was carried out as described previously by Lancashire and Mattoon (4).

Media

The following media were used: YPAD (complex glucose) - 1% yeast extract, 1% peptone, 2% glucose, 100 mg/liter adenine sulfate. YPGal (complex galactose) 1% yeast extract, 1% peptone, 2% galactose.

Cytochrome spectra

Strains were cultured to the end of logarithmic phase in YPAD or YPGal media containing Tween 80 (1.0%) and ergosterol (20 mg/l). δ-Aminolevulinic acid was added to a final concentration of 0.5 mM when required. After washing, cell suspensions were adjusted to a concentration of exactly 24 mg dry wt of cells/ml. Absolute or difference spectra were recorded in a split-beam scanning spectrophotometer using either glucose or sodium dithionite as reductant as necessary. Dry weight was determined gravimetrically using Gelman filters to collect and wash cells.

Table 1. Yeast Strains Employed

Strain	Genotype		Source
GT5-5D	*a lys2 trp2 cyd1*	ρ^{+}	Woods *et al.* (2)
GT5-5D/AA7	*a lys2 trp2 cyd1*	ρ°	*EBr treatment of GT5-5D
JC11/AA2	α *his4 kar1*	ρ°	Lancashire and Mattoon (4)
E3	*a ade lys*	ρ^{+} *oxi2*	Philip S. Perlman
CD27	α *his4 kar1*	ρ^{+} *oxi2*	Cytoductant from cross E3 x JC11/AA2
CD31	*a lys2 trp2 cyd1*	ρ^{+} *oxi2*	Cytoductant from cross GT5-5D/AA7 x CD27

*EBr - ethidium bromide

RESULTS

Introduction of *oxi2* mitochondria into a *cyd1* strain by cytoduction

Construction of a *cyd1* strain containing mitochondria bearing the *oxi2* mutation was accomplished in two steps using cytoduction (4). The *oxi2* mitochondria were first transferred from Strain E3 to a ρ° strain carrying the nuclear gene *kar1* (Strain JC11/AA2). After the two strains had been allowed to mate, initial buds from several zygotes were set apart by micromanipulation and both buds and remaining zygotes were allowed to form clones. Clones were selected which contained the nuclear phenotype of strain JC11 and exhibited the colony morphology characteristic of strains carrying the *mit*$^-$ mitochondrial marker (slightly creamy color). Comparison of the whole-cell spectrum of one of these clones with that of Strain E3 verified the presence of the ρ^+ *oxi2* genome (Fig. 1). The clone therefore represented a cytoductant, Strain CD27, with the JC11 nucleus and the mutant (*oxi2*) mitochondrial genome of Strain E3. In the second step, Strain CD27 was mated with *cyd1*ρ° Strain GT5-5D/AA7 and first-buds were again separated from zygotes by micromanipulation. Among 29

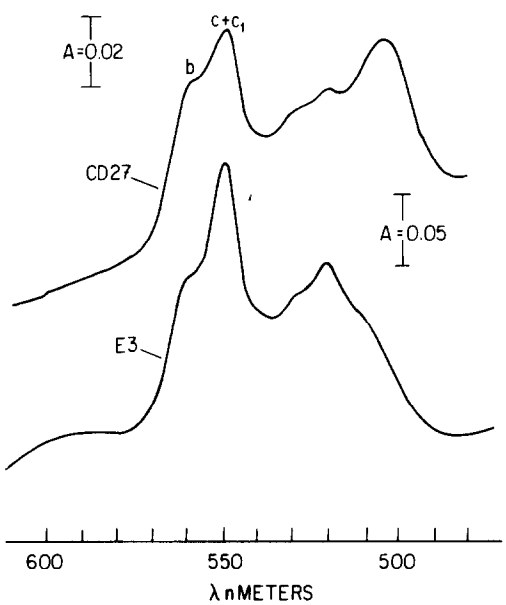


Figure 1. Whole-cell cytochrome spectra of *oxi2* donor strain E3 and its cytoductant CD27.

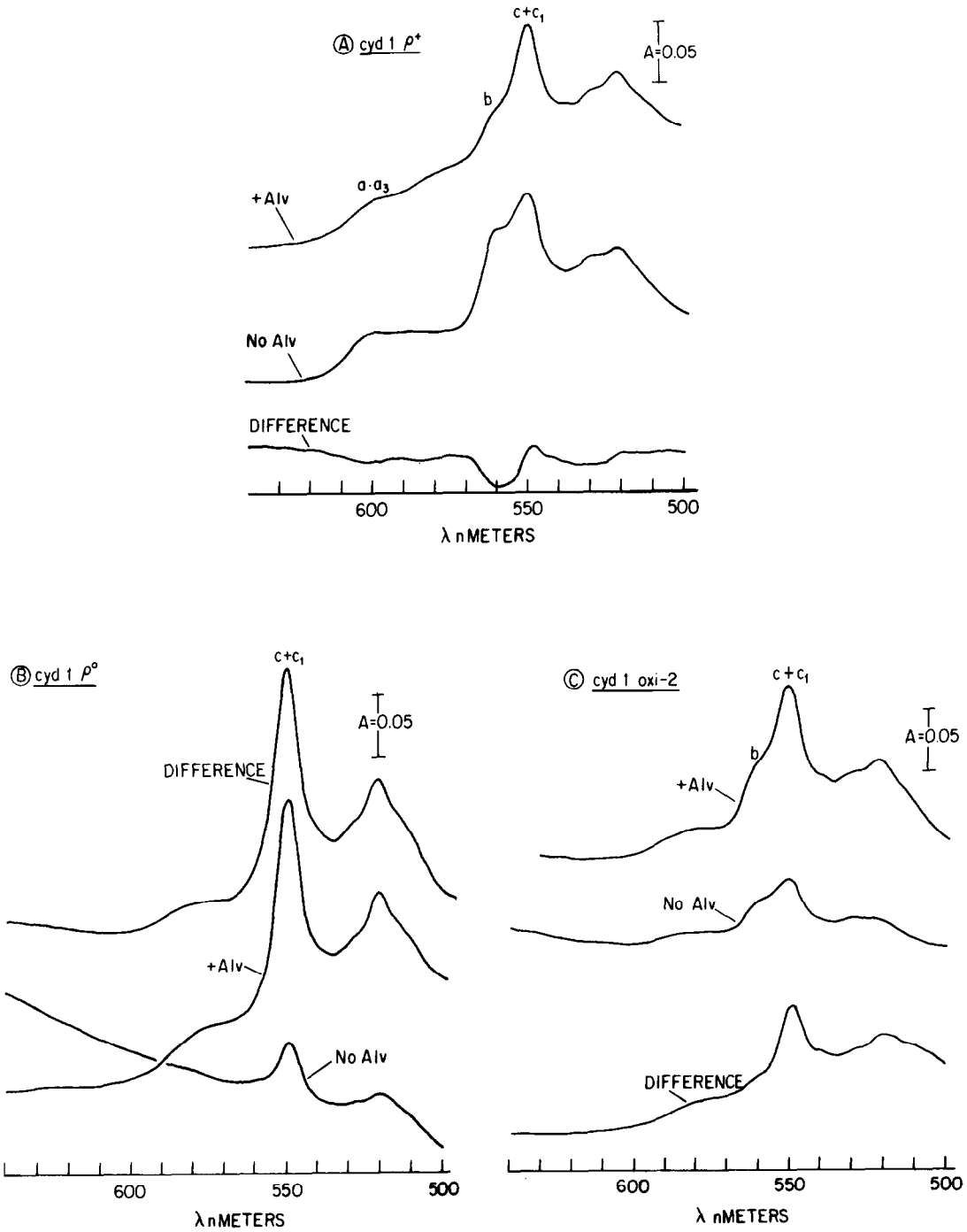


Figure 2. Whole-cell spectra of isonuclear derivatives of Strain GT5-5D grown with and without Alv. A. ρ^+ B. ρ° C. *oxi2* cytoductant. Cells were grown on 2% glucose medium (YPAD).

zygote- and first-bud-clones, 7 *oxi2* cytoductants were identified. A single cytoductant clone was chosen, and after purification by subcloning was designated as Strain CD31 (*cyd1 oxi2*). With this strain it was then possible to examine the behavior of the nuclear mutation *cyd1* in three isonuclear strains bearing different mitochondrial genotypes: ρ^+ (normal), ρ° and ρ^+*oxi2*.

Unmasking of *cyd1* cytochrome deficiency by ρ° and *oxi2*

Fig. 2 illustrates the unmasking effects of ρ° and *oxi2* on the cytochrome deficiency associated with the *cyd1* gene. It should be stressed that these experiments were made with cells grown on medium containing 2% glucose as carbon source. When mitochondria bearing the normal ρ^+ genome (Panel A) are present in the cell, the cytochrome spectrum appears to be normal, and addition of δ-aminolevulinic acid (Alv) to the growth medium causes only a small increase in cytochrome *c* and in a putative Zn-porphyrin (5) band which absorbs at about 583 nm. In contrast, when cells are in the ρ° state, cytochrome *c* is barely detectable (Panel B, curve 2) and is greatly stimulated by addition of Alv to the growth medium. The behavior of the cytoductant (*cyd1 oxi2*) is illustrated in Panel C of Fig. 2. As in the ρ° strain, cytochrome production is quite low in unsupplemented medium, but the unmasking effect is not as marked as with ρ°. The Alv-dependent increment in cytochromes is also less than in the *cyd1*ρ° strain. It appears, then, that the unmasking of the *cyd1* defect in ρ° strains is mainly due to deficient respiration and probably does not indicate the action of a specific mitochondrial regulatory gene (1).

Effects of derepression on expression of the *cyd1* defect in ρ^+ cells

Normal yeast cells grown on galactose media exhibit a higher cytochrome content than cells cultivated on glucose media. Comparison of Fig. 3 with Fig. 2A shows that this derepression effect of galactose is also evident in the *cyd1*ρ° Strain GT5-5D. Accompanying this derepression is a *partial* unmasking of the latent *cyd1* defect, since addition of Alv to growth medium now results in a significant enhancement of cytochrome synthesis. This effect is clearly revealed by the difference spectrum in Fig. 3. Apparently the

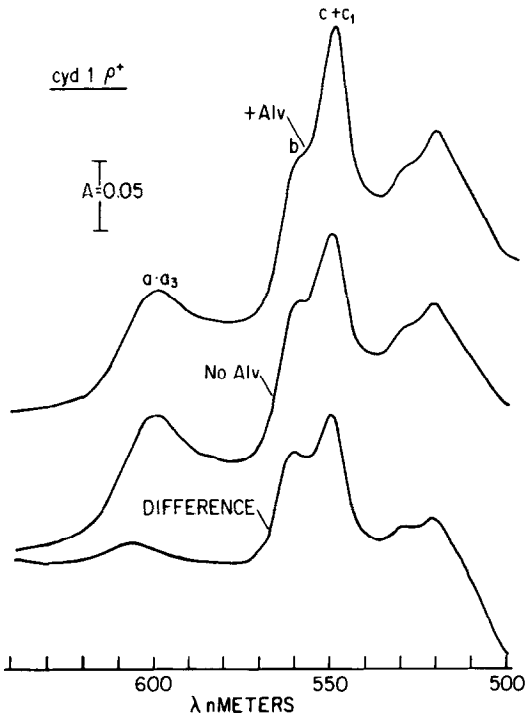


Figure 3. Whole-cell spectra of Strain GT5-5D grown on galactose medium with and without Alv.

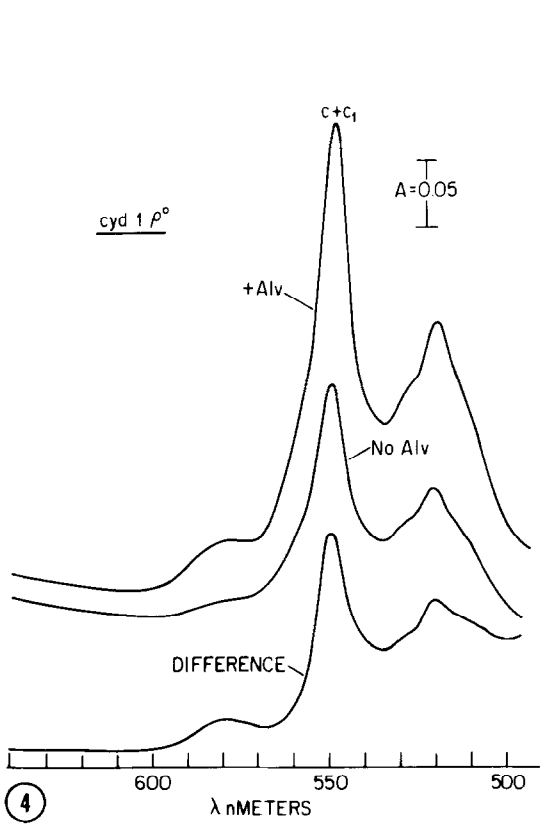
increased cytochrome synthesis in derepressed cells increases the demand for porphyrin biosynthesis sufficiently to exceed the capacity of the abnormal Alv synthase in the *cyd1* strain and Alv synthase activity becomes rate-limiting.

Effects of derepression on cytochrome and porphyrin synthesis in *cyd1*ρ° cells

The derepression effect of galactose is very pronounced in *cyd1*ρ° cells (compare Fig. 4 with Fig. 2B). Derepression reveals that *cyd1*ρ° cells do possess a substantial capacity to produce Alv synthase. Nevertheless the defective nature of this enzyme is revealed by the fact that Alv supplementation greatly increases cellular levels of cytochrome *c*. It also increases production of the putative Zn porphyrin absorbing at about 580 nm.

Derepression of *cyd1 oxi2* cells

Comparison of Fig. 5 with Fig. 3 reveals that the production of *b*- and *c*-type cytochromes is very strongly enhanced by the *oxi2* mutation when cells



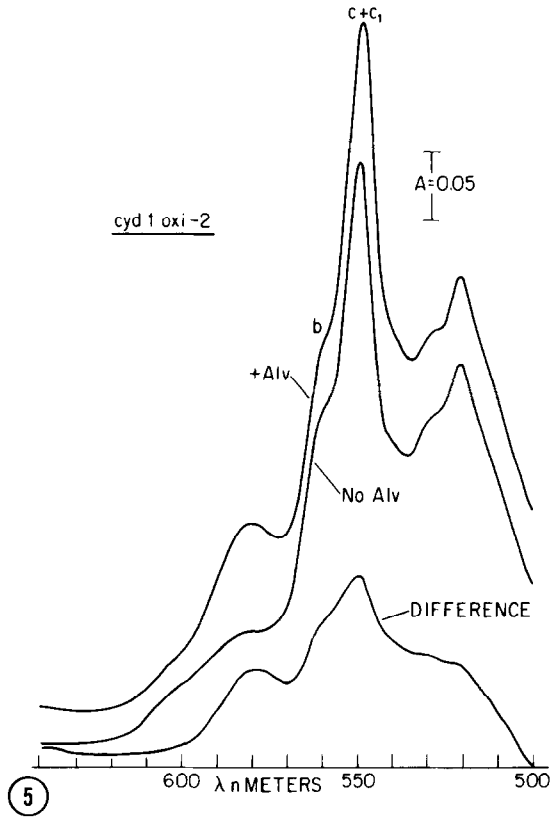


Figure 4. Whole-cell spectra of a ρ° derivative of GT5-5D (GT5-5D/AA7) grown on galactose medium with and without added Alv.

Figure 5. Whole-cell spectra of an *oxi2* cytoductant of GT5-5D/AA7 grown on galactose medium with and without added Alv.

are grown on galactose medium. Zn porphyrin and cytochrome synthesis can be increased still further by adding Alv. These results are also consistent with the suggestion that cells contain an altered Alv synthase which does not function as efficiently as the normal enzyme.

DISCUSSION

This study shows that respiratory deficiency is sufficient to unmask the latent defect in cytochrome formation associated with *cyd1*. Since extreme cytochrome deficiency occurs in glucose-grown cells, but not when galactose is used, glucose repression appears essential for its expression.

The mitochondrial mutation *oxi2* in a *cyd1* strain causes substantial decrease in cytochromes which is restored by added Alv. This unmasking is similar to but less extreme than that caused by the ρ° mutation. Since *oxi2* is a "point" mutation, it is unlikely that unmasking by ρ° involves loss of a *specific* mitochondrial gene which regulates Alv synthase activity or formation (1). Unmasking by ρ°, as first described (1) had two aspects: 1) an extreme cytochrome deficiency, 2) Alv-dependent cytochrome formation. Both aspects are obtained with glucose but not with galactose as carbon source. In brief, extreme cytochrome deficiency is glucose-dependent. Partial Alv dependence, in contrast, is observed with either carbon source.

In *cyd1* Alv *production* is rate-limiting for heme synthesis. This limited Alv formation probably reflects a structural defect in Alv synthase. The substantial cytochrome synthesis obtained in ρ° and *oxi2* strains grown on galactose indicates that neither mitochondrial mutation severely damages the mutant enzyme. However, in glucose, these same mutations severely limit Alv production. Alv synthase in normal ρ^+ yeast undergoes some decrease when cells are grown in glucose medium (6-8). Moreover, the enzyme in resting ρ^- cells is almost completely inactivated by glucose (9). Thus it appears to be in a highly vulnerable state in respiration-deficient cells when glucose is present. Alv synthase activity in *cyd1* extracts is very low, indicating that the mutant enzyme is abnormally labile (2). The following hypothesis is therefore proposed to explain *cyd1* unmasking: Alv synthase undergoes a normal degradation which is favored by glucose. Mutation to ρ° or *oxi2* augments this process. Since Alv synthase of *cyd1* is labile, it is almost completely lost in respiration-deficient cells placed in glucose medium.

Another observation of interest is the extremely high levels of *b*- and *c*-type cytochromes found in derepressed *oxi2* cells grown with Alv. Apparently porphyrin normally destined for heme *a* is used to produce abnormal amounts of other cytochromes. Zn porphyrin accumulation in Alv-supplemented *oxi2* is indicated by the substantial absorption near 580 nm.

This project illustrates the great utility of cytoduction (4) for manipulating mitochondrial genomes independently of nuclear genomes. In this case, we avoided problems of varying nuclear genetic backgrounds in evaluating mitochondrial genome effects on *cyd1* expression.

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