Low-Temperature Induction of Respiratory Deficiency in Yeast Mutants†

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ABSTRACT: Three cold sensitive mutants were isolated from a haploid strain of *Saccharomyces cerevisiae*. These mutant strains display a restricted capacity for growth at 18° on nonfermentable carbon sources. During aerobic growth on galactose at 18°, the respiratory capacity of the mutants decreases to less than 15% of the 28° level. The cytochrome content of the mutant strains, which is depressed with respect to wild type even in cells grown at 28°, decreases further after growth at 18°. The loss of respiration following growth at 18° is reversible, as incubation of nongrowing cells at 28° results in a restoration of respiratory capability. From the ability of two of the strains tested to carry out respiratory adaptation at 18° when first grown anaerobically at 28°, we conclude that some steps involved in the conversion of promitochondria to mitochondria are not in common with the temperature-induced adaptation. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of mitochondria obtained from cells grown at 28° and labeled with [^{3}H]leucine at 28° in the presence of cycloheximide reveals major alterations in the profile of products of mitochondrial protein synthesis; some of these alterations are amplified further in cells grown and labeled at 18°. Analysis of diploids obtained from crosses of two of the cold sensitive strains into (1) a grande and (2) a petite which lacks mitochondrial DNA, shows that cold sensitivity in both cases is recessive. These results are interpreted to be indicative of a nuclear locus or loci for cold sensitivity.

A number of different classes of mutants with defective or altered mitochondrial properties have been isolated from *Saccharomyces cerevisiae*. Among these are included the well characterized highly pleiotropic cytoplasmic petite (Ephrussi, 1953; Avers *et al.*, 1964; Mounolou *et al.*, 1966; Grossman *et al.*, 1969; Fukuhara *et al.*, 1969; Hollenberg *et al.*, 1969), segregational petites or *pet* mutants (Sherman, 1963; Sherman and Slonimski, 1964; Reilly and Sherman 1965), and cytoplasmically inherited mutants resistant to inhibitors of mitochondrial protein synthesis (Linnane *et al.*, 1968; Bunn *et al.*, 1970; Coen *et al.*, 1970) or to inhibitors of mitochondrial energy metabolism (Avner and Griffiths, 1970). Recently we described the isolation of cold sensitive mutants in yeast which displayed a restricted capacity for growth at 18° on nonfermentable carbon sources (Weislogel and Butow, 1970). In one particular strain, characterized as *genotypically* unstable with regard to mitochondrial continuity, cytoplasmic petites were efficiently produced during growth at low temperature or by growth at 28° in the presence of inhibitors of mitochondrial protein synthesis.

The rationale we have adopted for the selection and characterization of cold sensitive mutants with putative defects in mitochondrial function is that the reactions involving the assembly of cell structures should show a strong temperature dependence so that improper assembly of a functional respiratory chain might be manifested as cold sensitive mutations. As a continuation of this approach, we report here the isolation and partial characterization of cold sensitive mutants in which the mitochondrial genome is stable during growth at 18°, but which nevertheless lose respiratory capability at low temperature and thus appear *phenotypically* unstable. These strains are capable of carrying out mitochondrial protein synthesis, and the present study was undertaken to examine, in part, the effects of the mutations on products of mitochondrial protein synthesis.

Methods and Materials

Growth Conditions. Liquid and solid media (2% agar) contained 1% Difco yeast extract, 1% Difco Bactopeptone, 0.1% KH_2PO_4, and 0.12% $(NH_4)_2SO$ supplemented with 1% ethanol, 2% glucose, 2% galactose, or 3% glycerol, and designated as YPE, YPG, YPGal, and YPGly, respectively. Cells were grown aerobically in a rotary water bath shaker at 18 or 28°. Growth was recorded as optical density in a Klett photometer using a no. 66 (red) filter. Anaerobic cultures were grown in liquid medium supplemented with 5% glucose, 0.1% ethanol, 0.003% ergosterol, and 0.1% Tween 80.

Strains. S. cerevisiae strains 650-2C (α, *trp*$^-$, and *his*$^-$) and 55-R5-3C (a, *ur*$^-$) were obtained from Dr. P. Slonimski. Strains 24, 49, and 518-4 were derived from 650-2C by the following procedure. Strain 650-2C was grown on YPGal at 28° to log phase. An aliquot was harvested, washed once with sterile H_2O suspended in 10 ml of sterile H_2O and placed in a petrie dish. Following ultraviolet (uv) irradiation (to about 1% survivors), the contents of the petrie dish were transferred to YPGly and allowed to grow at 28° for about 4 hr. The cultures were then shifted to 18° and growth was continued for another 16 hr. Mycostatin sterile powder (Squibb) (Snow, 1966) was then added to a final concentration of 50 μg/ml, and growth was continued at 18°. Aliquots were removed after 1 and 2 hr, washed twice with sterile H_2O, suspended in H_2O, and plated out on YPG. After 24-hr incubation at 28°, replica plates were made on YPG and YPE and incubated at 28 and 18°. Mutants were selected for their inability to grow after 4 days on YPE plates at 18°. The strains were further purified by recloning.

Scoring and Isolation of Petites. The percentage of petites in a culture was determined by plating cells on differential media containing 3% glycerol and 0.1% glucose as described by

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Sherman (1967). Plates were scored visually as grandes or petites after 4-days growth at 28°.

Labeling of Cells. Cells to be labeled for gel electrophoresis were grown to early stationary phase in YPGal medium at either 28 or 18°. After harvesting, the cells were washed twice with cold distilled water and resuspended at 1 g wet wt of cells/ml of a medium containing 50 mM KPO_4 (pH 7.4), 0.3% glucose, 20 μg/ml of tryptophan, and 10 μg/ml of histidine. Cycloheximide was added to a final concentration of 600 μg/ml. After 15-min incubation at either 28 or 18° (cells were incubated at the same temperature at which they were grown), 10 μCi/ml of L-[^{14}C]leucine (250 Ci/mol, New England Nuclear) or 80 μCi/ml of L-[^{3}H]leucine (50 Ci/mol, New England Nuclear) was added. After 1 hr (28°) or 2 hr (18°) of incubation with shaking, 5 mg/ml of cold leucine was added to each flask. Incubation was continued for an additional 10 min and the flasks were rapidly chilled on ice. The cells were washed twice with cold distilled water containing 1 mg/ml of leucine. ^{3}H- and ^{14}C-labeled cells were combined and resuspended at 1 g wet wt of cells/2.5 ml of a solution containing 250 mM mannitol, 20 mM Tris-SO_4 (pH 7.4), 1 mM EDTA, and 1 mg/ml of leucine (MTEL).

Isolation of Mitochondria. Cells suspended in MTEL were subjected to two 30-sec shakings in a CO_2-cooled Bronwill MSK grinder using 10 g of 0.5-mm diameter glass beads in a 15-ml shaker flask. The suspension was decanted and the beads were rinsed with four 5-ml aliquots of MTEL. The supernatant suspension and rinses were combined and centrifuged twice at 1500*g* for 10 min to complete removal of unbroken cells and cell debris. The supernatant solution was centrifuged at 10,000*g* for 20 min to obtain the crude mitochondrial pellet. The mitochondria were washed once in MTEL and further purified by isopycnic banding on a 30–70% linear sucrose gradient. The final mitochondrial pellet was resuspended at a protein concentration of about 5 mg/ml in the mannitol–Tris–EDTA buffer described above minus the leucine.

Gel Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of labeled mitochondrial proteins was carried out as previously described (Weislogel and Butow, 1971). The gels were sliced after freezing, dissolved by treatment with 1 N NH_4OH overnight and counted in Aquasol (New England Nuclear). After correcting for background and spillover, the amount of ^{14}C and ^{3}H present in each slice was expressed as a percentage of the total ^{14}C or ^{3}H counts recovered from the gel.

Analytical Methods. Whole cell respiration measurements were made polarographically at 23° with a Clark oxygen electrode (Yellow Springs Instrument Co.) in 1.2 ml of medium containing 50 mM phosphate buffer (pH 7.4) and 1% ethanol (designated as O_2 electrode buffer). Whole cell spectra were recorded at the temperature of liquid nitrogen in a medium containing 50 mM phosphate buffer (pH 7.4) and 50% glycerol. Protein was determined by the method of Strickland (1951) or Lowry *et al.* (1951).

Results

Growth Rates. Table I compares the effects of temperature and carbon source on the aerobic growth rates of the parental stock 650-2C, and three independently isolated mutant strains. It is seen that at 28°, the growth rates of the mutants and parental strain are similar to each other and differ by less than a factor of 2 regardless of the carbon source used. At 18°, the growth rates are also comparable when fermentable carbon sources such as glucose or galactose are used, but are most severely depressed in the mutant strains when ethanol serves as substrate.

TABLE I: Effect of Temperature and Carbon Source on Aerobic Growth Rate.[a]

Temp (°C)	Strain	Mean Generation Time (hr)		
		Glucose	Galactose	Ethanol
28	650-2C	1.9	3.6	3.6
	24	1.6	3.2	6.0
	518-4	2.0	3.3	4.0
	49	2.0	2.9	4.6
18	650-2C	5.4	6.5	7.4
	24	7.8	7.5	27
	518-4	5.2	7.3	21
	49	5.2	8.0	23

[a] Each strain was grown at 28 or 18° on either YPG, YPGal, or YPE as indicated. At various times, Klett values were obtained to determine growth rates.

Effect of Growth Temperature on Whole Cell Respiratory Rates. Since functional mitochondria are required in yeast for growth only on nonfermentable carbon sources, the markedly decreased ability of the mutant strains to grow at 18° on ethanol medium in contrast to the glucose and galactose media would suggest a loss of mitochondrial function. Consistent with this view is the fact that when cultures of the cold sensitive strains growing on 2% galactose are shifted from 28 to 18°, respiratory capability is rapidly lost. As shown in Figure 1B, after about 2 generations at 18° the rate of whole cell respiration in strain 24 falls to only 15% of the 28° level; respiratory capability decreases at a somewhat slower rate in strains 518-4 and 49. In contrast to these results, respiration in the parental strain is unaffected by this regime.

We have previously reported a similar phenomenon in another cold sensitive strain of *S. cerevisiae* (Weislogel and Butow, 1970). In this case, respiratory capability was also rapidly lost during growth at 18° and this loss could be completely accounted for by the efficient production of cytoplasmic petites, *i.e.*, the mitochondrial genome in this strain is unstable at the low temperature. This explanation does not apply, however, in the case of the cold sensitive strains reported here. As shown in Figure 1A, the percentages of petites present in cultures as a function of growth at 18° are not significantly different from the values obtained in cultures grown at 28° while clearly, respiration has decreased more than 10-fold in the mutant strains after 6–8 doublings at 18°. The respiratory capability of cells of the mutant strains, previously grown at 28°, is unaffected by incubation at 18° when carried out under conditions of nongrowth.

Temperature-Induced Respiratory Adaptation. The fact that a large percentage of the cells present in cultures grown at 18° are scored as grandes indicates that the phenomenon of low temperature induced respiratory decrease is reversible. To document this point, strains 24, 518-4, 49, and 650-2C were grown on galactose for about 3–4 generations at 18°. The cells were then washed and resuspended in adaptation medium (50 mM phosphate buffer (pH 7.4), 1% ethanol, 0.1% glucose, 10 μg/ml of histidine, and 20 μg/ml of tryptophan) and incubated at 28°. At various time intervals aliquots were re-

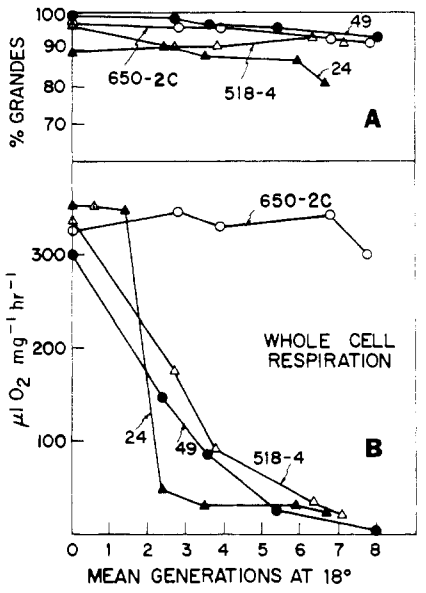

FIGURE 1: (A) Percentage of respiratory competent cells (Grandes) as a function of growth at 18°. All strains were grown on YPGal at 28°; at zero-time cultures were then shifted to 18°. Aliquots were removed at the indicated times, diluted with sterile distilled water, and plated on differential media and incubated at 28° as described in Methods and Materials. When necessary, cells were transferred to fresh YPGal medium to maintain the cultures in log phase. (B) Loss of respiratory capacity during growth at 18°. Aliquots were removed from the above cultures, washed twice with cold water and suspended in O_2 electrode buffer to a protein concentration of 3–10 mg/ml and assayed for respiratory activity as indicated.

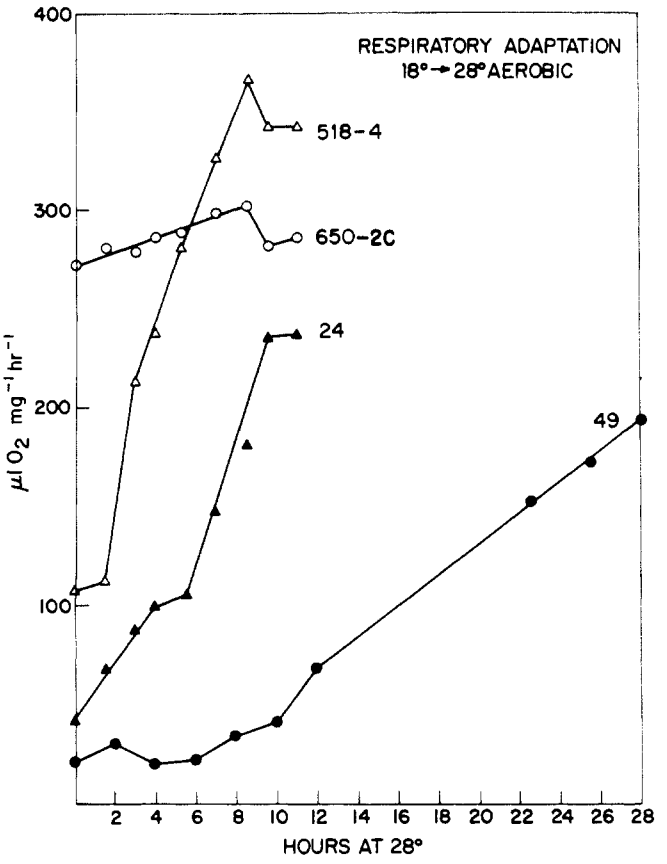

FIGURE 2: Recovery of respiratory capacity in a nongrowth medium at 28°. Cells were grown at 18° for 3–4 generations. Aliquots were removed and respiratory rates were determined. The remaining cells were harvested, washed twice with water, and resuspended in adaptation medium (50 mM phosphate buffer (pH 7.4), 1% ethanol, 0.1% glucose, 10 μg/ml of histidine, and 20 μg/ml of tryptophan) to the same cell density prior to harvesting. The cells were then aerated at 28° and aliquots were removed at various times for respiratory measurements. During adaptation, the cells were supplemented to 0.2% glucose every 2 hr.

moved and the respiratory rates were determined. Figure 2 shows that respiratory capability does, indeed, recover at 28° in a manner analogous to the classical respiratory adaptation of repressed yeast cells (Slonimski, 1953). The temperature adaptation phenomenon shown here, like respiratory adaptation in response to oxygen and lowered glucose concentration, can occur under conditions of nongrowth. Cells maintained in adaptation medium at 18° adapt to only about 5% of the 28°-induced level of adaptation.

As illustrated for strain 24, respiratory adaptation in response to temperature requires products of both mitochondrial and cytoplasmic protein synthesis since the process is inhibited by both cycloheximide and chloramphenicol (Table II). Moreover, the petite-inducing mutagen ethidium bromide, a reagent which selectively inhibits mitochondrial RNA synthesis (Penman *et al.*, 1970; Fukuhara and Kujawa, 1970), also inhibits the high-temperature-induced respiratory adaptation. An 8-hr incubation with ethidium bromide significantly depressed respiration in the parental strain, 650-2C. This observation may reflect some long-term secondary effects of ethidium of bromide on mitochondrial respiration.

Effect of Temperature on Respiratory Adaptation of Anaerobically Grown Cells. Some insight into the block in the development or expression of functional mitochondria which occurs during growth at 18° could be obtained by examining the temperature dependence of the conversion of promitochondria to mitochondria. If cells of the cold sensitive mutants grown anaerobically at 28° are capable of carrying out respiratory adaptation at 18° as well as at 28°, the expression of mitochondrial phenotype which is cold sensitive probably lies at some step or steps not in common with those involved in the conversion of promitochondria into functional mitochondria. Figure 3 demonstrates that strains 518-4 and 24 are indeed capable of adapting at 18° although, as expected, the rate is slower than at 28°. The overall extent of adaptation for 24 and 518-4 at 18° is about the same as for the parental strain 650-2C (about 3-fold), although the adaptation rate for the mutants is somewhat less than that for the parent at either temperature. The effect of temperature on the adaptation rate of strain 49 was not tested.

Whole Cell Spectra. An analysis of the low-temperature spectra obtained from cultures of the parental and mutant strains grown at 28 and 18° is shown in Figure 4. First, it should be noted that in cells of the parental strain 650-2C grown at 18°, less cytochrome aa_3 (λ_{max} at about 600 nm) is present than in cultures grown at 28°. This result confirms our previous observations on the apparent decrease in cytochrome oxidase content in wild-type yeast when grown at low temperature on nonrepressing, fermentable carbon sources (Weislogel and Butow, 1970). Second, the decrease in cytochrome aa_3 content noted here is evidently insufficient to significantly restrict the respiratory capability of strain 650-2C after extended growth at 18° (Figure 1B). In the cold sensitive strains, the cytochrome oxidase content as well as the content of cytochromes *b* and c_1 is diminished with respect to 650-2C, in cultures grown at 28°, but nevertheless, respiratory rates remain high. Growth at 18°, however, results in a further de-

TABLE II: Effect of Inhibitors on Respiratory Adaptation: 18–28°.[a]

Strain	Additions	μl of O_2/ mg per hr
650-2C (zero time)	None	209
650-2C (8 hr at 28°)	None	230
650-2C (8 hr at 28°)	Chloramphenicol (3 mg/ml)	214
650-2C (8 hr at 28°)	Cycloheximide (600 μg/ml)	254
650-2C (8 hr at 28°)	Ethidium bromide (10 μg/ml)	144
24 (zero time)	None	45
24 (8 hr at 28°)	None	192
24 (8 hr at 28°)	Chloramphenicol (3 mg/ml)	49
24 (8 hr at 28°)	Cycloheximide (600 μg/ml)	76
24 (8 hr at 28°)	Ethidium bromide (10 μg/ml)	53

[a] Strains 650-2C and 24 were grown for five generations at 18° on YPGal. The cells were harvested, washed twice with water, and suspended to the same cell density in 50 mM phosphate buffer (pH 7.4), containing 1% ethanol and 0.1% glucose. Aliquots were removed for measurements of whole cell respiration. Chloramphenicol, cycloheximide, or ethidium bromide was added to zero time as indicated. After 8-hr incubation with shaking at 28°, aliquots were removed for respiration measurements. The medium was supplemented to 0.2% glucose every 2 hr.

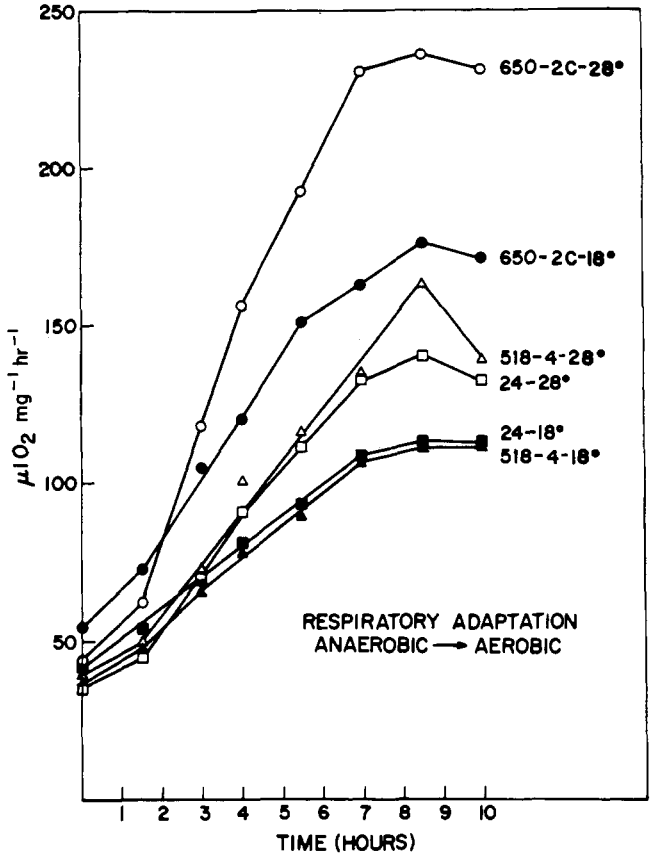

FIGURE 3: Effect of temperature on respiratory adaptation of anaerobically grown cells. Cells were grown in sealed flasks in anaerobic medium (Methods and Materials) which was deaerated by bubbling with O_2-free nitrogen passed through a 10% solution of alkaline pyrogallic acid. The cells were grown to stationary phase, harvested, washed twice with cold water, and suspended in adaptation medium and incubated at 28 or 18°. Respiratory adaptations were monitored as described in Figure 2.

crease in the content of cytochrome *b* and c_1, and a decrease of cytochrome oxidase levels to nearly nondetectable amounts. This could account for the precipitous drop in whole cell respiration following a 28–18° shift particularly for strains 24 and 49, and, in effect, amplifies the phenotypic differences between the mutant strains and the wild type.

Products of Mitochondrial Protein Synthesis. The incorporation of labeled amino acid into products of mitochondrial protein synthesis results in the appearance of a small number of polypeptide species on sodium dodecyl sulfate–polyacrylamide gels (Weislogel and Butow, 1971). We have compared the gel profile of this limited number of components in both 18 and 28° grown cultures of strains 24 and 49 with the gel profile obtained from strain 650-2C, grown and labeled under identical conditions. These results are illustrated in Figures 5 and 6. In cultures of 650-2C grown at 28°, the labeling profile is similar to that which we have previously reported (Weislogel and Butow, 1971) for products of mitochondrial protein synthesis. It is clearly evident, however, that even in cultures of the cold sensitive strains grown and labeled at 28° pronounced alterations in the gel pattern, compared to that of 650-2C, are observed. In particular, relative to the total counts recovered on the gel, some products of mitochondrial protein synthesis appear depleted, others are unchanged or increased in amount, while other components appear slightly shifted to lower molecular weight. Some of these differences are even more evident in the gel patterns obtained from mitochondrial isolated from cells grown and labeled at 18°. Most

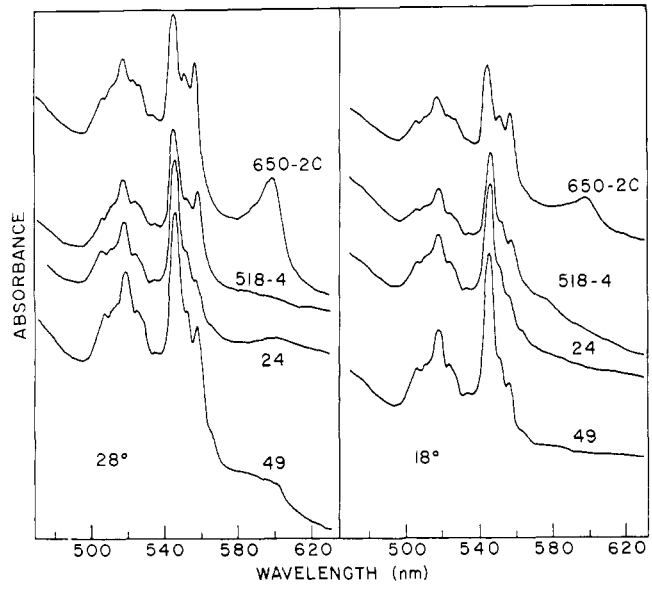

FIGURE 4: Whole cell spectra at the temperature of liquid nitrogen. Cells were grown on YPGal at 18 or 28° to stationary phase. Samples were harvested, washed twice in cold distilled water, and suspended in 0.1 volume of 50% glycerol–50 mM phosphate buffer (pH 7.4). Samples were frozen in liquid nitrogen and the spectra were recorded on a dual-wavelength spectrophotometer.

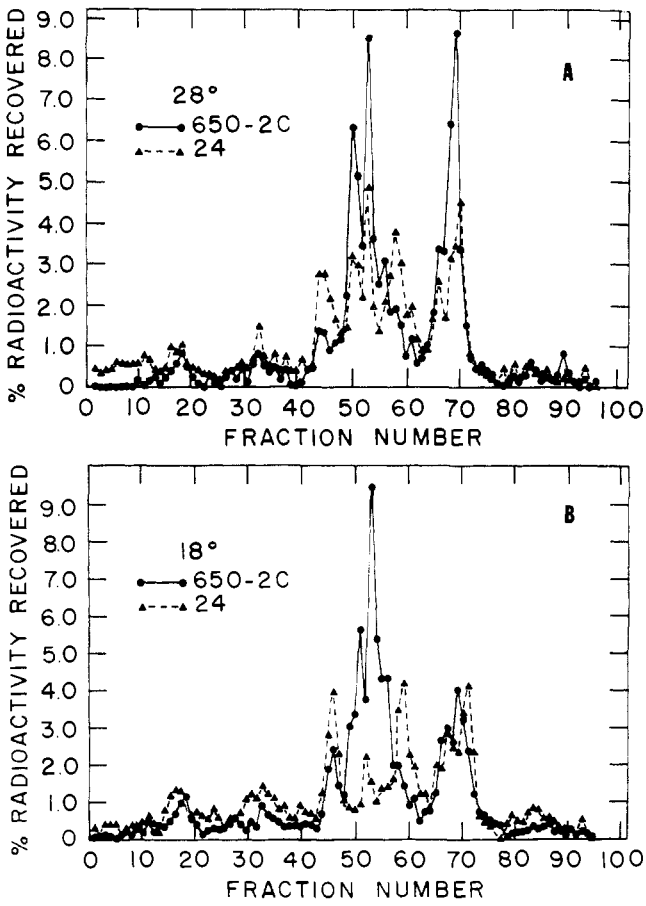

FIGURE 5: Double-label sodium dodecyl sulfate–gel profiles of mitochondria isolated from 650–2C and 24. Cells were grown at 28° (A) or 18° (B) on YPGal harvested in early stationary phase, and labeled for 1 hr (28°) or 2 hr (18°) in the presence of 600 μg/ml of cycloheximide. Mitochondria were isolated and analyzed on sodium dodecyl sulfate gels as described in Methods and Materials. 650-2C, [^{14}C]leucine; 24, [^{3}H]leucine.

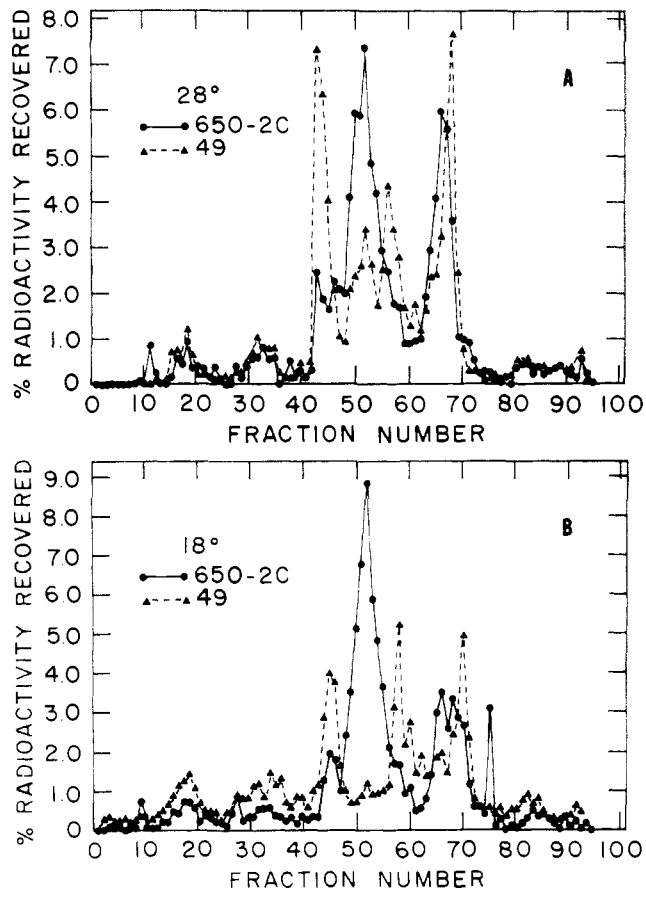

FIGURE 6: Double-label sodium dodecyl sulfate–gel profiles of mitochondria isolated from 650-2C and 49. Growth, labeling, and analysis of labeled mitochondrial proteins were the same as described in Figure 5. (A, 28°; B, 18°); 49, [^{3}H]leucine; 650-2C, [^{14}C]leucine.

notably, the material between fractions about 48–56 (Figures 5B and 6B) is almost entirely absent in the cold sensitive strains. From our previous analysis of the molecular weight distribution of these products of mitochondrial protein synthesis (Weislogel and Butow, 1971), this material corresponds to molecular weights in the range from 34,000 to 44,000.

TABLE III: Comparison of Respiratory Activity of Diploids Grown at 18°.[a]

Diploid	natoms of O_2/min per mg
650-2C X 55-R5-3C	320
49 X 55-R5-3C	246
24 X 55-R5-3C	204
650-2C X 183	204
49 X 183	172
24 X 183	181

[a] Diploids of the crosses indicated were grown at 18° in 20 ml of YP Gal medium. Cells were harvested in early stationary phase, washed once in water, and resuspended in about 1 ml of O_2 electrode buffer. Aliquots of suspensions were assayed for respiratory activity. Strain 183 which lacks mitochondrial DNA is a petite derived from strain 55-R5-3C.

Genetic Origin of the Cold Sensitive Phenotype. A tetrad analysis of the segregation of cold sensitive respiratory capacity has been carried out on strains 24 and 49. These experiments did not yield a simple 2:2 segregation pattern characteristic of a single nuclear gene mutation. However, some indication of the genetic origin of the mutations, *i.e.*, nuclear or cytoplasmic, could be obtained by comparing the respiratory capacities, after growth at 28 and 18°, of diploids constructed from crosses of the cold sensitive and parental strains with (1) a wild-type ρ^+ strain (grande) and (2) a ρ^- strain (petite), otherwise isogenic with the wild-type strain above, but which lacks mitochondrial DNA. For these experiments, we used a ρ^- strain (183), which we have previously shown to lack mitochondrial DNA (Weislogel and Butow, 1971). Recent evidence indicates that petites which lack mitochondrial DNA are neutral regarding suppression of ρ^+ function (Michaelis *et al.* 1971) so that the mitochondrial phenotype of diploids constructed from these strains and ρ^+ cells would be expected to reflect solely the contribution of the ρ^+ partner. The data in Table III show that diploids obtained from crosses of strains 24 and 49 with a ρ^- strain (55-R5-3C) or the ρ^- isogenic derivative 183, which lacks mitochondria DNA, have comparably high respiratory rates after growth at 18°. These results suggest a nuclear locus or loci for the cold sensitive respiratory capacity in strains 24 and 49.

Discussion

We have now described two classes of cold sensitive mutants in yeast which show altered mitochondrial properties. In earlier reports, (Weislogel and Butow, 1970, 1971), we demonstrated that in one particular cold sensitive strain, loss of mitochondrial function at the "nonpermissive" temperature is the result of hypermutability to cytoplasmic petites. In the strains described here, however, the marked reduction in the ability of cells to grow on nonfermentable carbon sources at 18°, along with a concomitant loss of respiratory capability, represents a reversible loss of mitochondrial function. To a certain extent, the strains described in this report resemble some nuclear petites or *pet* mutants (Sherman, 1963; Sherman and Slonimski, 1964; Reilly and Sherman, 1965). Like the latter, the amounts of cytochromes *b* and c_1, and particularly aa_3, are reduced in comparison to wild-type strains, in cells of the cold sensitive mutants grown even at 28°. Nevertheless, the cold sensitive strains display high rates of respiration when grown at 28° and have the capacity to grow at that temperature on nonfermentable carbon sources.

Our results on whole cell spectra show that growth of wild-type yeast at 18° partially restricts the amount of cytochrome oxidase present. As a result, when the cold sensitive strains that are already deficient in cytochrome oxidase when grown at 28° are grown out at 18°, cytochrome oxidase levels are reduced still further to amounts not detectable spectrally. This result could account for the restricted capacity of these strains to grow at 18° on nonfermentable carbon sources. In addition, loss of respiratory capability would be expected as the terminal respiratory chain apparatus becomes rate limiting. These results serve to amplify the temperature-dependent synthesis or assembly of a functional terminal respiratory chain system in yeast mitochondria.

Our results also suggest that some further resolution of the events leading to the assembly of functional mitochondria in yeast may be possible. This is seen by the ability of cold sensitive strains grown anaerobically at 28° to carry out respiratory adaptation at 18°; yet, these strains are still unable to develop respiratory capacity when grown aerobically at 18° and can only do so in a temperature shift of 28°. Hence, the steps involved in the conversion of promitochondria (defined here as those mitochondrial precursors which accumulate in cells grown anaerobically at 28° on high glucose concentrations) to mitochondria are not cold sensitive.

Fully repressed yeast cells are completely lacking in cytochromes of the mitochondrial electron-transport chain (Lindenmayer and Estabrook, 1958). By this criterion, cells of the cold sensitive strains grown at 18° are, in a sense, not as fully repressed as anaerobically grown yeast; nevertheless, as shown in Table II for strain 24, adaptation at 28° requires products of both mitochondrial and cytoplasmic protein synthesis. Since the conversion of promitochondria to mitochondria also requires cytoplasmic and mitochondrial translation products (Fukuhara, 1965), and since it is possible to adapt anaerobically grown cold sensitive strains at 18°, our data would indicate that the conversion of promitochondria to mitochondria proceeds through some steps not in common with those of the temperature induced adaptation. These observations raise the question as to what extent the process of the development of functional mitochondria from promitochondria shares events in common with those involved in the continued biosynthetic activities associated with the maintenance of mitochondrial continuity during non-fermentative, logarithmic growth of yeast. While it is not possible to resolve this question at the moment, it is of interest that although products of mitochondrial protein synthesis are indispensable for the conversion of promitochondria to mitochondria, only a small fraction of the total protein synthetic capacity of promitochondria appears to be "turned on" during respiratory adaptation (Groot *et al.*, 1972). What is also of particular interest is the fact that the alterations in products of mitochondrial protein synthesis reported here in cells which still contain considerable amounts of cytochromes of the mitochondrial respiratory chain are much greater relative to the pattern seen with wild-type yeast than in a similar comparison between promitochondria which completely lack respiratory chain cytochromes and fully developed functional mitochondria (Groot *et al.*, 1972).

It is possible to draw some conclusions correlating the effects of the cold sensitive mutations and the content of respiratory carriers with the synthesis of products of mitochondrial protein synthesis: a mutation or mutations altering the levels of mitochondrial respiratory carriers but not necessarily causing their complete elimination results in striking alterations in the synthesis or possible integration into the inner mitochondrial membrane of polypeptides synthesized on mitoribosomes. When cells are placed under conditions (*e.g.*, 18°) which further depress the cytochrome content, a number of major polypeptide species are nearly absent from the gel profiles. This is seen in a comparison of the sodium dodecyl sulfate–gel patterns of mitochondria from cells grown at 28 and 18°. Other components seem to be relatively unaffected by this regime. At this point, it is not possible to say if those components which seem to be shifted in position relative to the wild-type pattern represent modified or incomplete polypeptides or rather an amplification, as a result of the normalization procedure, of minor species whose synthesis is relatively unaffected in the mutants.

Recently Thomas and Williamson (1971) reported pronounced alterations in the electrophoretic pattern of products of mitochondrial protein synthesis in a temperature-sensitive mutant of yeast unable to grow on nonfermentable carbon sources at 35°. In their communication, no report was given of the effects of high temperature on the cytochrome content in the mutant or on the possible effect of temperature alone on the relative distribution of label into the various polypeptide components. That some temperature dependence of the relative amounts of label incorporated into the various polypeptide species synthesized on mitoribosomes occurs is evident from the experiments reported here. Those components in the gel profiles of mitochondria from the parental strain 650-2C between fractions 60-75 (Figures 5 and 6) represent a much larger percentage of the total incorporated material in the 28° experiments than in those at 18°. Clearly then, significant alterations in products of mitochondrial protein synthesis can occur under a variety of experimental conditions. Some of these alterations may be directly related to changes in cytochrome content while others may reflect perturbations in components not associated with electron-transport activity.

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Utilization of Exogenous Fatty Acids for Complex Lipid Biosynthesis and Its Effect on *de Novo* Fatty Acid Formation in *Escherichia coli* K-12†

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ABSTRACT: The utilization of exogenous fatty acids by wild-type *Escherichia coli* K-12 has been studied using [^{14}C]acetate incorporation to monitor fatty acid synthesis and unusual fatty acid analogs as a nonradioactive supplement to distinguish acyl groups in the phospholipid derived from the exogenous source. Certain strains are found to regulate synthesis of saturated and unsaturated fatty acids in response to exogenous fatty acid supplements. This effect is associated with incorporation of the exogenous supplement into phospholipid. Even and odd chain-length saturated, trans and cis unsaturated fatty acids were included among the fatty acid supplements examined. Although a principal result of the incorporation of the exogenous fatty acid is a replacement of structurally related acyl groups, the effect is pleotropic in many instances: for example, 16:0 replaces 18:1 and *vise versa*. These observations can be rationalized in terms of the positional distribution in the phospholipid of the acyl residues derived from synthesis and the pattern of incorporation of the exogenous fatty acids. β-OH-14:0 and -12:0, added to cultures as supplements, are not detectably incorporated into complex lipid (would be predominantly lipid A) and show little or no influence on fatty acid synthesis.

Recent observations (Esfahani *et al.*, 1971; Sinensky, 1971; Silbert *et al.*, 1972) have demonstrated that certain strains of *Escherichia coli* K-12 are capable of regulating unsaturated fatty acid biosynthesis in response to an unsaturated fatty acid supplement in the medium. When *cis*-Δ^9-octadecenoate is added to the culture, *cis*-Δ^{11}-octadecenoate and *cis*-Δ^9-hexadecenoate synthesis are markedly reduced together with a small decrease in the production of tetradecanoate and hexadecanoate (Silbert *et al.*, 1972). It is thought that the specificity of the response may reflect the extent to which the exogenous unsaturated fatty acid can replace various endogenously derived fatty acyl groups in the complex lipids (Silbert *et al*,, 1972). In the present study saturated, cis and trans unsaturated, and β-hydroxy fatty acids are tested as supplements in exponentially growing cultures of the two strains which had demonstrated regulation of unsaturated fatty acid synthesis in the previous study. The results clearly show a relationship between the regulation of fatty acid synthesis by exogenous fatty acids and the fashion and extent to which the external fatty acids are incorporated into phospholipid.

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