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# *PET* Genes of *Saccharomyces cerevisiae*

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## INTRODUCTION

*Saccharomyces cerevisiae* is a facultative anaerobic yeast capable of satisfying its energy requirements with the ATP made from fermentation. The nonessentiality of respiration for viability makes this organism ideal for genetic and biochemical dissections of the processes responsible for the maintenance of functional mitochondria. Earlier genetic and molecular characterizations of yeast mitochondrial DNA led to a fairly detailed understanding of the contribution of this genome to the propagation of a respiratory-competent organelle (2, 41, 181). Even though the number of mitochondrial gene products is small, they are critical for the expression of respiratory competence because of their catalytic functions in electron transport and in oxidative phosphorylation. As a result of more recent efforts in many laboratories, there is a rapidly increasing body of information about the dependence of the respiratory potential of yeasts on a vast array of genes located in the nucleus (43, 44, 83, 154). Many of these new data have emerged from studies of respiratory-deficient mutants of *S. cerevisiae*. An understanding of the biochemical lesions incurred by such mutants should eventually provide a blueprint of how the mitochondrial organelle is made.

In this article we have cataloged characterized nuclear genes that affect the respiratory capacity of *S. cerevisiae*. This information is drawn from several sources. First, we describe genes that have been identified from studies of mutants obtained in our laboratory. Second, we have compiled genes whose products have been shown by others to be necessary for respiration. Whenever possible, these genes have been related by various means to complementation groups in the collection described here. Finally, we include genes whose products are housed in mitochondria but are not necessary for respiration.

## DEFINITION OF *PET* GENES AND MUTANTS

When grown on media containing glucose, respiratory-defective mutants of *S. cerevisiae* form smaller colonies than wild-type cells do. This morphological feature is a consequence of the inability of such strains to metabolize ethanol produced from glucose. The growth of mutant, but not of wild-type cells, therefore, is arrested once glucose is exhausted in the medium. The term cytoplasmic *petite* mutant was used to describe this characteristic of respiratory-defective strains with cytoplasmically inherited mutations (47). These strains were later shown to have long deletions in mitochondrial DNA or to completely lack the organellar genome (60, 61, 111). To distinguish cytoplasmic *petite* mutants from respiratory-deficient strains with genetic lesions in nuclear genes, the latter are commonly referred to as nuclear *petite* or *pet* mutants (114, 161). Like cytoplasmic *petite* mutants, growth of *pet* mutants is limited by the availability of fermentable substrates (163).

In the context of the present discussion, the term *pet* mutant will be applied to any strain of *S. cerevisiae* which, as a result of a mutation in nuclear DNA, loses the ability to utilize nonfermentable but not fermentable carbon sources. According to this definition, *pet* mutants are substrate conditional but may, in addition, have growth properties conditional on other environmental factors. For example, the inability of a *pet* mutant to grow on a nonfermentable substrate may be temperature dependent. It should be noted that as defined here, *pet* mutants are identified solely on the basis of their growth phenotype without any prejudice as to the type of function that may be affected. This definition is not without problems, some of which will be addressed below.

Although most mutations leading to the growth phenotype of *pet* mutants define gene products that either are directly involved in the oxidative metabolism of mitochondria or are necessary for the expression of this activity, there are exceptions. A case in point involves mutations in certain gluconeogenic enzymes required for the conversion of non-sugar substrates to glucose, which is necessary for cell wall biosynthesis. Such mutants do not grow on nonfermentable substrates and have an apparent respiratory-defective phenotype even though the oxidative and phosphorylative capacity of their mitochondria is normal (146, 155).

Consequent to the definition of a *pet* mutant, a *PET* gene is a nuclear gene having at least one mutant allele that will confer a respiratory-deficient phenotype. Until recently the wild-type genes were capitalized and distinguished by number, e.g., *PET1*, *PET2*. The mutant genes were designated by

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TABLE 1. Selection of *pet* mutants

| Mutagen | No. of groups | Selection | Parental strain(s) | Reference |
|---|---|---|---|---|
| Sodium nitrite | ? | Negative on glycerol (nonconditional) | SM11-6C, FM8α | 135 |
| Ethyl methanesulfonate and nitrosoguanidine | 34 | tetramethyl-*p*-phenylenediamine reduction (nonconditional) | JM22 | 106 |
| Ethyl methanesulfonate | 12 | Negative on glycerol and ethanol (nonconditional) | D273-10B | 43, 44 |
| Ethyl methanesulfonate | 8 | Negative on glycerol at 36°C, positive on glycerol at 22°C | 124 | 115 |
| Ethyl methanesulfonate | 11 | Negative on glycerol at 35°C, positive on glycerol at 25°C, extensive conversion to *rho*$^-$ after 3 days at 35°C | D273-10B | 58 |
| Ethyl methanesulfonate | 106 | Negative on lactate at 36°C, positive on lactate at 22°C | X2180-1A, S2180-1B | 14 |
| UV, ethyl methanesulfonate nitrosoguanidine | 11 | Negative on glycerol, normal composition of cytochromes (nonconditional) | D311-3A | 129 |

lowercase letters with the allele number separated by a dash, e.g., *pet1-1*, *pet2-1*. Even though some investigators still adhere to this convention, most new genes for which a function can be assigned are designated by a descriptive three-letter symbol consistent with the convention used to name other yeast nuclear genes. Whenever possible we will use *PET* and *pet* as generic terms to indicate a particular type of gene.

## ISOLATION OF *pet* MUTANTS

Most collections of nuclear respiratory-defective mutants, including the one described here, are composed of strains selected for their ability to grow on glucose but not on a nonfermentable substrate such as glycerol or lactate (Table 1). Such strains are conveniently recognized by their colony morphology after plating of a mutagenized stock on medium containing a limiting concentration of glucose (0.1 to 0.2%) and a high concentration of the nonfermentable substrate. On this type of medium, respiratory-defective strains with mutations in either nuclear or mitochondrial DNA give rise to small colonies for reasons stated above. To distinguish nuclear mutants from the more abundant class of mitochondrial (cytoplasmic *petite*) mutants, the strains are crossed to a yeast tester lacking mitochondrial DNA (*rho*$^0$) and the diploid progeny are checked for growth on a nonfermentable substrate. Growth of the diploid cells indicates that the respiratory defect is caused by a recessive mutation in a nuclear gene. Conversely, mutants whose respiratory defects are not complemented by the *rho*$^0$ tester have mutations or deletions in mitochondrial DNA. It is important to note that this test does not distinguish strains whose respiratory defects are due to mutations in mitochondrial DNA from mutants that may have an additional mutation in a *PET* gene. This protocol can also be used to isolate temperature-sensitive *pet* mutants (14, 115).

Collection of respiratory-deficient strains selected only for the above differential growth properties on the two substrates ensures mutations in the most diverse assortment of *PET* genes. Mutants affected in a narrower range of functions can be obtained by other selections. Temperature-conditional *pet* mutants which undergo loss of wild-type mitochondrial DNA at the nonpermissive temperature will be enriched for lesions in gene products necessary for mitochondrial DNA replication, protein synthesis, transcription, and some aspects of RNA processing (34, 115). Mutants can also be preselected for the loss of a particular mitochondrial cytochrome component by spectroscopic means (162) or by replica platings on media containing redox dyes that mediate electron transport in confined segments of the respiratory chain (106, 107). Parental strains with mutations in certain genes have also been used to develop selection schemes for mutants defective in particular types of functions. For example, mutations abolishing respiration are lethal in strains lacking the constitutively expressed alcohol dehydrogenase gene (18). This fact allows for enrichment of mutations in tricarboxylic acid cycle enzymes (19). Mutations in certain kinds of *PET* genes can also be obtained by isolation of suppressors in different mitochondrial or nuclear respiratory-deficient mutant strains. Such suppressors have been obtained for mutations in mitochondrial rRNA genes (9, 10, 22, 73, 168), in the apocytochrome *b* and/or subunit 1 of cytochrome oxidase genes (23, 40, 64, 152), in subunit 3 of cytochrome oxidase (40), in the ATPase subunit 9 gene (70), in the 3′ processing site of *VAR1* (206), in mitochondrial import presequences (8), and in the *RPO41* gene coding for a subunit of the mitochondrial RNA polymerase (95, 96, 109). Only a few of the suppressors, however, have been characterized (7, 71, 89, 94, 95, 152).

The range of gene functions represented in a collection of *pet* mutants depends not only on the selection procedure but also on the criteria used to score growth or lack thereof on the nonfermentable substrate and the mitochondrial and nuclear genetic backgrounds of the parental respiratory-competent strain. The importance of some of these factors is illustrated by the following few examples. Mutations that impair functions such as mitochondrial protein synthesis result in a secondary loss of wild-type mitochondrial DNA due to the acquisition of long deletions (119). The extent to which the wild-type mitochondrial genome is lost from a *pet* mutant culture is a function of the effectiveness of the mutational block. The tighter the mutation, the more rapid and quantitative is the disappearance of normal mitochondrial DNA from the population. There are two ways in which this particular problem can be circumvented. One is to isolate strains whose growth on the nonfermentable substrate is compromised but not totally abolished. Alternatively, mutations in this subset of *PET* genes can be obtained through isolation of temperature-sensitive strains.

There are trivial situations in which the mitochondrial genetic background of the parental strain will exclude the detection of mutations in certain genes. The absence in some mitochondrial genomes of introns whose processing depends on nuclear gene products will prevent the expression of a respiratory-defective phenotype when such proteins are inactivated (156). There are also occasions when the severity of a respiratory-deficient phenotype may be affected by the nuclear genetic background. For example, on rich glycerol medium, commonly used for the analysis of respiratory-defective strains, tricarboxylic acid cycle mutants show a wide range of growth phenotypes. The same mutant allele

TABLE 2. *PET* complementation groups

| Group | Mutant[a] | Total no. of mutants[b] | Clone[c] | Gene name | Gene product or phenotype | Sequence obtained[d] | Deposited[e] | Reference(s)[f] |
|---|---|---|---|---|---|---|---|---|
| G1 | C103 | 46 | + | *ATP2* | β subunit of $F_1$ ATPase | C | | 151, 173, 179 |
| G2 | C146 | 62 | + | | Coenzyme $QH_2$-cytochrome *c* reductase deficient | P | | A |
| G3 | C296 | 32 | + | *COQ1* | Hexaprenyl pyrophosphate synthetase | C | | B |
| G4 | C4 | 72 | + | | Cytochrome oxidase deficient | C | | D |
| G5 | C5 | 3 | + | *FUM1* | Mitochondrial and cytoplasmic fumarase | C | ATCC, YC | 200 |
| G6 | C6 | 26 | + | *LIP1* | Lipoic acid deficient | C | | E |
| G7 | C153 | 22 | + | *COR1* | 45-kDa subunit of coenzyme $QH_2$-cytochrome *c* reductase | C | ATCC, YC | 183 |
| G8 | C8 | 2 | + | | Cytochrome oxidase deficient | C | | F |
| G9 | C9 | 10 | − | *COQ4* | Coenzyme Q deficient | | | |
| G10 | C33 | 26 | − | *COQ2* | Hexaprenyl pyrophosphate transferase | | | B |
| G11 | C145 | 7 | + | *MSK1* | Mitochondrial lysyl-tRNA synthetase | C | | G |
| G12 | C13 | 25 | + | *RIP1* | Rieske protein of coenzyme $QH_2$-cytochrome *c* reductase | C | | 5 |
| G13 | C15 | 14 | + | *ATP11* | Assembly of $F_1$ ATPase | C | | H |
| G14 | C17 | 2 | − | | Cytochrome oxidase deficient | | | |
| G15 | C18 | 13 | − | | Pleiotropic[g] | | | |
| G16 | C19 | 8 | + | | Cytochrome oxidase deficient | | | |
| G17 | C83 | 20 | + | *COQ5* | Coenzyme Q deficient | | | |
| G18 | C141 | 7 | − | | Pleiotropic | | | |
| G19 | C28 | 37 | + | *COX10* | Cytochrome oxidase deficient (homolog of ORF1 in *Paracoccus denitrificans* cytochrome oxidase operon) | C | | I |
| G20 | C23 | 3 | − | | Heme deficient | | | |
| G21 | C100 | 13 | − | | Normal[g] | | | |
| G22 | C25 | 5 | + | | Cytochrome oxidase deficient | | | |
| G23 | C26 | 18 | + | *PET494* | COXIII mRNA translation factor | C | | 24, 43, 116 |
| G24 | C27 | 4 | − | | Pleiotropic | | | |
| G25 | C30 | 17 | − | | Normal | | | |
| G26 | C31 | 1 | + | *MSY1* | Mitochondrial tyrosyl-tRNA synthetase | C | | J |
| G27 | C34 | 5 | − | | ATPase deficient | | | |
| G28 | C35 | 10 | + | *CBP3* | Coenzyme $QH_2$-cytochrome *c* assembly factor | C | ATCC, YC | 201 |
| G29 | C279 | 7 | + | | Pleiotropic | C | | F |
| G30 | E125 | 8 | + | | Pleiotropic | C | | K |
| G31 | C39 | 41 | − | *COQ3* | 3,4-Dihydroxy-5-hexaprenylbenzoic acid methylase | | | 59, 166 |
| G32 | C41 | 5 | + | *HEM2* | δ-Aminolevulinate dehydratase | C | ATCC, YC | 62, 117 |
| G33 | C43 | 1 | − | | Normal | | | |
| G34 | C46 | 14 | + | | Cytochrome oxidase deficient | | | |
| G35 | C47 | 3 | − | | Cytochrome oxidase deficient | | | |
| G36 | 89 | 27 | + | *CBP2* | Intron bI5 splicing factor | C | ATCC, YC | 56, 108 |
| G37 | C155 | 3 | + | *MEF1* | Mitochondrial elongation factor (homolog of procaryotic elongation factor G) | C | | L |
| G38 | C50 | 8 | + | | Pleiotropic | | | |
| G39 | C51 | 2 | − | | Pleiotropic | | | |
| G40 | C52 | 2 | − | | Pleiotropic | | | |
| G41 | C171 | 8 | + | *SCO1* | Cytochrome oxidase deficient | C | | 153 |
| G42 | C54 | 22 | − | | Heme deficient | | | |
| G43 | C55 | 41 | + | *OP1* | ATP-ADP exchange carrier | C | | 1, 4, 84, 126 |
| G44 | C59 | 1 | − | | Normal | | | |
| G45 | C139 | 22 | + | *CYT2* | Cytochrome $c_1$ heme lyase | | | M |
| G46 | C106 | 18 | + | *COX5*a | Subunit Va of cytochrome oxidase | C | | 32, 79, 158 |
| G47 | C62 | 3 | + | | Cytochrome oxidase deficient | | | |
| G48 | C251 | 6 | − | | Pleiotropic | | | |
| G49 | C249 | 9 | − | *LIP3* | Lipoic acid deficient | | | |
| G50 | C198 | 43 | + | *ATP1* | α subunit of $F_1$ ATPase | C | | 172 |
| G51 | C69 | 13 | − | | Normal | | | |
| G52 | C70 | 5 | − | | Pleiotropic | | | |
| G53 | C164 | 14 | + | *COX11* | Cytochrome oxidase deficient (homolog of ORF3 in *P. denitrificans* cytochrome oxidase operon) | C | | N |
| G54 | C75 | 2 | + | *MRP4* | Mitochondrial ribosomal protein (homolog of *Escherichia coli* S2) | C | | F |
| G55 | C76 | 2 | + | *SDH1* | Succinate dehydrogenase deficient | | | |
| G56 | C79 | 1 | − | | Pleiotropic | | | |
| G57 | C264 | 16 | + | *ATP12* | $F_1$ assembly factor | C | | Bowman[h] |
| G58 | C119 | 41 | − | | Cytochrome oxidase deficient | | | |
| G59 | C151 | 17 | + | *MSL1*, *NAM2* | Mitochondrial leucyl-tRNA synthetase | C | ATCC, YC | 71, 88, 178 |

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TABLE 2—*Continued*

| Group | Mutant[a] | Total no. of mutants[b] | Clone[c] | Gene name | Gene product or phenotype | Sequence obtained[d] | Deposited[e] | Reference(s)[f] |
|---|---|---|---|---|---|---|---|---|
| G60 | C116 | 29 | + | *CBP1* | 5′ end processing factor for cytochrome *b* pre-mRNA | C | ATCC, YC | 36, 37 |
| G61 | C92 | 7 | − | | Normal | | | |
| G62 | C94 | 7 | − | | Pleiotropic | | | |
| G63 | C96 | 18 | − | *COQ6* | Coenzyme Q deficient | | | |
| G64 | C97 | 28 | − | *COQ7* | Coenzyme Q deficient | | | |
| G65 | C101 | 3 | − | | Normal | | | |
| G66 | C102 | 4 | + | *COX4* | Subunit IV of cytochrome oxidase | C | | 80, 99 |
| G67 | C105 | 5 | + | *COR4* | 14-kDa subunit of coenzyme $QH_2$-cytochrome *c* reductase | C | | 30, 33, 189 |
| G68 | C108 | 3 | − | | Normal | | | |
| G69 | C110 | 10 | + | *MST1* | Mitochondrial threonyl-tRNA synthetase | C | ATCC, YC | 128 |
| G70 | C225 | 6 | + | *KGD1* | α-Ketoglutarate dehydrogenase | C | ATCC, YC | 143 |
| G71 | C121 | 20 | + | *COX6* | Subunit VI of cytochrome oxidase | C | | 80, 199 |
| G72 | C134 | 8 | + | *MSM1* | Mitochondrial methionyl-tRNA synthetase | C | ATCC, YC | 182 |
| G73 | C125 | 8 | + | | Cytochrome oxidase deficient | | | |
| G74 | C129 | 1 | − | | Cytochrome oxidase deficient | | | |
| G75 | C130 | 40 | − | *COQ8* | Coenzyme Q deficient | | | |
| G76 | C246 | 2 | − | | Normal | | | |
| G77 | C212 | 22 | + | *PET111* | COXII mRNA translation factor | C | | 43, 140, 169 |
| G78 | C142 | 8 | + | | Pleiotropic | | | |
| G79 | C143 | 12 | + | *MSS116* | COXI pre-mRNA processing factor | C | | 156, 157 |
| G80 | C154 | 14 | − | | Cytochrome oxidase deficient | | | |
| G81 | E135 | 4 | − | | Normal | | | |
| G82 | C149 | 3 | + | | Pleiotropic | C | | O |
| G83 | C156 | 13 | + | *PET54* | COXIII mRNA translation factor | C | | 25, 26, 187 |
| G84 | E206 | 2 | − | | Normal | | | |
| G85 | C158 | 4 | − | | Pleiotropic | | | |
| G86 | C312 | 11 | + | *LIP2* | Lipoic acid deficient | P | | E |
| G87 | C161 | 2 | − | | Normal | | | |
| G88 | C162 | 1 | − | *HEM4* | Coproporphyrinogen decarboxylase | | | 62 |
| G89 | C167 | 1 | + | *MRP2* | Mitochondrial ribosomal protein (homolog of *E. coli* S14) | C | | 118 |
| G90 | P11 | 1 | − | | Normal | | | |
| G91 | C173 | 2 | + | | Cytochrome oxidase deficient | C | | F |
| G92 | C176 | 11 | + | | Cytochrome oxidase deficient | C | | F |
| G93 | C179 | 4 | + | | Cytochrome oxidase deficient | C | | Q |
| G94 | E234 | 2 | + | *MSD1* | Mitochondrial aspartyl-tRNA synthetase | C | ATCC, YC | 57 |
| G95 | N230 | 7 | + | | ATPase deficient | P | | H |
| G96 | C199 | 22 | + | *MSS51* | COXI pre-mRNA splicing factor | C | | 50 |
| G97 | C202 | 5 | + | | Cytochrome oxidase deficient | | | |
| G98 | C204 | 3 | + | | Normal | | | |
| G99 | C208 | 9 | + | | Pleiotropic | C | | F |
| G100 | E220 | 2 | + | | Pleiotropic | C | | M |
| G101 | C210 | 12 | + | *CYT1* | Cytochrome $c_1$ | C | | 30, 90, 150 |
| G102 | N174 | 11 | + | *CBP4* | Coenzyme $QH_2$-cytochrome *c* reductase assembly factor | C | | M |
| G103 | C220 | 5 | + | MSE1 | Glutamyl-tRNA synthetase | C | | F |
| G104 | E250 | 3 | + | *KGD2* | Dihydrolipoyl transsuccinylase | C | ATCC, YC | E |
| G105 | C229 | 1 | − | | Normal | | | |
| G106 | N1 | 3 | + | | Pleiotropic | C | | F |
| G107 | C235 | 6 | − | *LIP4* | Lipoic acid deficient | | | |
| G108 | E290 | 3 | | | Normal | | | |
| G109 | P16 | 1 | − | | Normal | | | |
| G110 | N160 | 2 | + | *PET56* | Pleiotropic | P | | F, 170 |
| G111 | C260 | 5 | + | | Cytochrome oxidase deficient | | | |
| G112 | C261 | 23 | + | *CYC3* | Cytochrome *c* heme lyase | C | | 42, 148, 162 |
| G113 | N205 | 3 | + | | ATPase deficient | C | | H |
| G114 | E177 | 9 | − | | Normal | | | |
| G115 | C287 | 2 | − | | COXI mRNA deficient | | | |
| G116 | C297 | 6 | − | | Pleiotropic | | | |
| G117 | N241 | 1 | + | | Pleiotropic | C | | F |
| G118 | N266 | 2 | + | | Cytochrome oxidase deficient | | | |
| G119 | C295 | 4 | + | | Normal | | | |
| G120 | C303 | 5 | + | *MSF1* | α subunit of mitochondrial phenylalanyl-tRNA synthetase | C | ATCC, YC | 81 |
| G121 | N328 | 4 | + | | Normal | | | |

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TABLE 2—*Continued*

| Group | Mutant[a] | Total no. of mutants[b] | Clone[c] | Gene name | Gene product or phenotype | Sequence obtained[d] | Deposited[e] | Reference(s)[f] |
|---|---|---|---|---|---|---|---|---|
| G122 | E221 | 6 | − | | Pleiotropic | | | |
| G123 | N356 | 2 | + | *CBS1* | Cytochrome *b* pre-mRNA and mRNA translation factor | C | | 82, 135, 145 |
| G124 | C317 | 1 | + | | Normal | | | |
| G125 | C318 | 3 | − | | Normal | | | |
| G126 | N363 | 2 | − | | Normal | | | |
| G127 | N380 | 2 | + | *MRP3* | Mitochondrial ribosomal protein (S19 homolog?) | C | | O |
| G128 | N393 | 8 | − | | Normal | | | |
| G129 | N413 | 1 | − | | Pleiotropic | | | |
| G130 | N415 | 1 | + | *TUFm* | Mitochondrial elongation factor (homolog of procaryotic factor EFTu) | C | | 121 |
| G131 | N420 | 2 | + | | Normal | | | |
| G132 | N472 | 2 | − | | Normal | | | |
| G133 | N518 | 1 | + | | Pleiotropic | C | | O |
| G134 | N520 | 4 | + | | Cytochrome oxidase deficient | | | |
| G135 | E282 | 3 | − | | Normal | | | |
| G136 | P37 | 1 | − | | Normal | | | |
| G137 | E384 | 4 | + | *FBP* | Fructose-1,6-bisphosphatase | C | | 146, 155 |
| G138 | P39 | 1 | − | | Normal | | | |
| G139 | E204 | 2 | + | | Pleiotropic | | | |
| G140 | E205 | 2 | + | | COXI mRNA deficient | | | |
| G141 | P48 | 1 | − | | Pleiotropic | | | |
| G142 | E252 | 4 | + | *MTF2* | Mitochondrial initiation factor (homolog of procaryotic factor IF2) | C | | F |
| G143 | E307 | 3 | + | *HAP3* | Nuclear transcription factor | C | | 68 |
| G144 | E354 | 4 | + | *COR2* | 40-kDa subunit of coenzyme $QH_2$-cytochrome *c* reductase | C | | 30, 127, 190 |
| G145 | E359 | 1 | − | | Cytochrome *c* deficient | | | |
| G146 | E203 | 1 | − | | Normal | | | |
| G147 | E214 | 1 | + | | COXI mRNA deficient | | | |
| G148 | E241 | 4 | − | | Pleiotropic | | | |
| G149 | E275 | 4 | + | *PET122* | COXIII mRNA translation factor | C | | 78, 106, 107, 125 |
| G150 | E411 | 7 | − | | Pleiotropic | | | |
| G151 | P65 | 1 | − | | Normal | | | |
| G152 | E123 | 1 | − | | Normal | | | |
| G153 | E145 | 2 | + | *COR5* | 11-kDa subunit of coenzyme $QH_2$-cytochrome *c* reductase | C | | 30, 98, 189 |
| G154 | E158 | 1 | + | *CBP6* | Cytochrome *b* mRNA translation factor | C | | 38 |
| G155 | E264 | 6 | − | | Normal | | | |
| G156 | P68 | 1 | − | | Normal | | | |
| G157 | E8 | 7 | − | | Cytochrome *c* deficient | | | |
| G158 | E39 | 2 | − | | Normal | | | |
| G159 | E59 | 5 | − | | Normal | | | |
| G160 | E57 | 1 | − | | Pleiotropic | | | |
| G161 | E64 | 1 | − | | Cytochrome oxidase deficient | | | |
| G162 | E67 | 5 | + | *CBP7* *CBS2* | Cytochrome *b* pre-mRNA and mRNA translation factor | C | | 135, 145, Muroff[i] |
| G163 | E96 | 5 | − | | Normal | | | |
| G164 | E280 | 1 | − | | Pleiotropic | | | |
| G165 | E103 | 3 | + | *ATP10* | ATPase deficient | C | ATCC, YC | H |
| G166 | E128 | 1 | − | | Normal | | | |
| G167 | E130 | 8 | − | | Normal | | | |
| G168 | E153 | 1 | − | | Pleiotropic | | | |
| G169 | E358 | 1 | − | | Pleiotropic | | | |
| G170 | E362 | 1 | − | | Normal | | | |
| G171 | E322 | 1 | − | | Pleiotropic | | | |
| G172 | E330 | 4 | − | | COXI mRNA deficient | | | |
| G173 | E343 | 1 | − | | Normal | | | |
| G174 | E372 | 2 | + | *MRF1* | Mitochondrial release factor | C | | S |
| G175 | E374 | 2 | − | | Pleiotropic | | | |
| G176 | P77 | 1 | − | | Normal | | | |
| G177 | E392 | 1 | + | | Pleiotropic | | | |
| G178 | P96 | 1 | − | | Normal | | | |
| G179 | E553 | 7 | − | | Normal | | | |
| G180 | E567 | 1 | − | | Pleiotropic | | | |
| G181 | E569 | 2 | + | *MSW1* | Mitochondrial tryptophanyl-tRNA synthetase | C | ATCC, YC | 120 |

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TABLE 2—*Continued*

| Group | Mutant[a] | Total no. of mutants[b] | Clone[c] | Gene name | Gene product or phenotype | Sequence obtained[d] | Deposited[e] | Reference(s)[f] |
|---|---|---|---|---|---|---|---|---|
| G182 | P104 | 1 | − | | Normal | | | |
| G183 | E602 | 2 | − | | Pleiotropic | | | |
| G184 | E530 | 2 | − | | Normal | | | |
| G185 | E649 | 1 | − | | Cytochrome *c* deficient | | | |
| G186 | P115 | 1 | − | | Normal | | | |
| G187 | E688 | 1 | − | | Pleiotropic | | | |
| G188 | E708 | 2 | − | | Cytochrome oxidase deficient | | | |
| G189 | E730 | 1 | + | | Pleiotropic | | | |
| G190 | E742 | 1 | − | | Normal | | | |
| G191 | E428 | 3 | − | | Cytochrome oxidase deficient | | | |
| G192 | E749 | 2 | − | | Pleiotropic | | | |
| G193 | E779 | 1 | − | | Pleiotropic | | | |
| G194 | E783 | 1 | − | | Normal | | | |
| G195 | E795 | 1 | + | *MRP1* | Mitochondrial ribosomal protein | C | | 118 |
| G196 | E827 | 2 | − | | Cytochrome oxidase deficient | | | |
| G197 | E838 | 1 | − | | Pleiotropic | | | |
| G198 | E847 | 2 | + | *ATP4* | Subunit 4 of ATPase | C | | 132, 191 |
| G199 | E880 | 1 | − | | Cytochrome oxidase deficient | | | |
| G200 | E884 | 1 | − | | Normal | | | |
| G201 | E885 | 2 | − | *KGD3* | Deficient in α-ketoglutarate dehydrogenase | | | |
| G202 | E887 | 2 | − | *PDH1* | Pyruvate dehydrogenase deficient | | | |
| G203 | E889 | 2 | − | | Normal | | | |
| G204 | E899 | 1 | − | | Pleiotropic | | | |
| G205 | P217 | 1 | − | | Pleiotropic | | | |
| G206 | E199 | 1 | − | | Pleiotropic | | | |
| G207 | E81 | 4 | + | *PET123* | Mitochondrial ribosomal protein(?) | C | | R |
| G208 | P235 | 1 | − | | Normal | | | |
| G209 | P272 | 1 | − | | Normal | | | |
| G210 | P274 | 1 | − | | Pleiotropic | | | |
| G211 | P287 | 1 | − | | Pleiotropic | | | |
| G212 | P302 | 1 | − | | Normal | | | |
| G213 | P311 | 1 | − | | Normal | | | |
| G214 | P317 | 1 | − | | Normal | | | |
| G215 | P403 | 1 | − | | Normal | | | |

[a] The mutants listed in this table were derived from *S. cerevisiae* D273-10B/A1 (177).
[b] Number of independent mutant isolates in the complementation group.
[c] +, The genes have been cloned; −, the genes are still not available.
[d] C, The gene has been completely sequenced; P, only a partial sequence has been obtained.
[e] Strains including the natural mutant, a mutant with either a disrupted or deleted copy of the gene, and a transformed strain harboring the wild-type gene on a multicopy plasmid have been deposited with the American Type Culture Collection, Rockville, Md., (ATCC) and the Yeast Genetic Stock Center, Berkeley, Calif. (YC). Strains of *E. coli* transformed with the wild-type yeast gene on episomal plasmids are also available from the American Type Culture Collection.
[f] Unpublished studies: A, Francisco Nobrega; B, Matthew Ashby; D, Ivor Muroff; E, Barbara Repetto; F, Alexander Tzagoloff; G, Domenico Gatti; H, Sharon Ackerman; I, Marina Nobrega; J, John Hill; K, Alexandra Gampel; L, Andrea Vambutas; M, Mary Crivellone; N, Nazzareno Capitanio; O, Alan Myers; Q, T. J. Koerner; R, Thomas Fox; S, Herman Pel.
[g] The term pleiotropic describes mutants in which all the cytochromes except cytochrome *c* are deficient. The term "normal" describes mutants with a normal complement of cytochromes.
[h] S. Bowman, Ph.D. thesis, University of Warwick, Coventry, England, 1989.
[i] I, Muroff, Ph.D. thesis, Columbia University, New York, N.Y., 1988.

may express a clear absence of growth in one strain and yet demonstrate near-wild-type growth in other genetic contexts (143, 200).

Several collections of *pet* mutants have been made over the last 20 years (Table 1). Similar to the mutants in the collection described in detail in this review, the mutants isolated by Parker and Mattoon (129), Ebner et al. (43, 44), and Pillar et al. (135) do not grow on glycerol at 30°C, the preferred growth temperature for *S. cerevisiae*. Individual constraints for two of these collections were (i) that the mutants also show no growth on ethanol (43, 44) and (ii) that the strains not be deficient in cytochromes (129). The most extensive collection listed in Table 1, that of Burkl et al. (14), is composed of 116 complementation groups of mutants that are temperature sensitive for growth on lactate. The more recent temperature-sensitive collections by Mueller et al. (115) and Genga et al. (58) contain mutants that not only are *pet* at the restrictive temperature, but also have a high rate of loss of the mitochondrial genome. The collection of McEwen et al. (106) is unique in that the design of the screen was meant to identify only *pet* mutants defective in cytochrome oxidase function. After mutagenesis, colonies that stained with tetramethyl-*p*-phenylenediamine, indicating cytochrome oxidase activity, were not retained.

## COMMENTS ON THIS MUTANT COLLECTION

All of the *pet* mutants listed in Table 2 were obtained by mutagenesis of the respiratory-competent haploid strain *S. cerevisiae* D273-10B/A1 (177) with either ethyl methanesulfonate or nitrosoguanidine (179, 180). Respiratory-deficient strains, ascertained by crosses to a cytoplasmic *petite* tester to have mutations in nuclear DNA, were purified and scored for their growth characteristics on rich glycerol medium. All

mutants, even those displaying a leaky phenotype on this medium, were kept as long as their growth could be distinguished from that of the wild type. Because of the somewhat permissive definition of what constitutes a respiratory-deficient mutant, the collection includes strains with mutations in genes such as those encoding mitochondrial ribosomal proteins, aminoacyl-tRNA synthetases, etc., that otherwise would have been lost because of the aforementioned relationship between the degree of loss of function (in this case mitochondrial protein synthesis) and stability of the wild-type mitochondrial genome.

Approximately 2,000 independent *pet* strains were obtained in six separate screens. Of these, 1,700 were assigned to the 215 complementation groups listed in Table 2. Although other mutants were also analyzed, the results of the crosses were not clear and an assessment of whether they represented new or already established complementation groups was difficult.

Owing to the complexity of this genetic system, the extent of saturation of the nuclear genome for *PET* genes cannot be estimated by standard statistical methods. There are reasons to think, however, that most *PET* genes are represented in the collection. First, each successive screen has yielded progressively fewer mutants defining new complementation groups. The first 400 strains analyzed had mutations in 100 different genes. The last screen, involving approximately the same number of isolates, yielded only eight new groups of mutants. Second, allelism tests have generally permitted the assignment of mutants from other laboratories to groups already existing in our collection.

The instability of mitochondrial DNA in the context of certain *pet* backgrounds means that not all genes will be equally represented by mutations. Thus, complementation groups defining gene products involved in mitochondrial protein synthesis have only a few members that tend to have leaky phenotypes. This should also apply to mutants defective in mitochondrial DNA replication and in transcription and processing of the endogenous rRNAs and tRNAs. In view of this circumstance, a disproportionately large number of complementation groups are composed of relatively few isolates.

## PHENOTYPIC CLASSES

Single representatives from most complementation groups have been assayed for NADH-cytochrome *c* reductase, cytochrome oxidase, oligomycin-sensitive ATPase, and mitochondrial protein synthesis. In addition, absorption spectra of mitochondrial cytochromes have been recorded. On the basis of these biochemical analyses, the mutants can be classified into one of the following phenotypic classes: (i) cytochrome oxidase-deficient mutants, (ii) coenzyme $QH_2$-cytochrome *c* reductase-deficient mutants, (iii) ATPase-deficient mutants, (iv) mutants impaired in mitochondrial protein synthesis as assayed by in vivo incorporation of radioactive precursors into mitochondrially translated proteins (this class is also pleiotropically deficient in cytochrome oxidase, coenzyme $QH_2$-cytochrome *c* reductase, and oligomycin-sensitive ATPase), and (v) mutants with a normal complement of respiratory-chain enzymes and ATPase.

Each of the above phenotypes describes a fairly broad range of nuclear gene products and functions. Nonetheless, knowledge of these phenotypes is helpful in limiting biochemical screens for lesions in a known component to mutants from a smaller number of complementation groups. It should be obvious that mutations in a mitochondrial aminoacyl-tRNA synthetase will produce a pleiotropic phenotype, whereas mutations in functionally important subunits of cytochrome oxidase are more likely to express a deficiency in this enzyme only. The search for specific mutants by biochemical means can therefore usually be confined to strains from one particular phenotypic class.

The types of biochemical lesions ascertained to induce the five different phenotypes (listed above) are briefly described, since they provide useful guidelines for biochemical screens.

(i) Most cytochrome oxidase-deficient strains also lack spectrally detectable cytochromes *a* and $a_3$. The range of functions affecting the synthesis of cytochrome oxidase is identical to that described for coenzyme $QH_2$-cytochrome *c* reductase. In addition, this phenotypic class should include mutations in enzymes of the heme *a* biosynthetic pathway, none of which are known at present.

(ii) The mutant class deficient in coenzyme $QH_2$-cytochrome *c* reductase is characterized by the absence of antimycin-sensitive coenzyme $QH_2$-cytochrome *c* reductase activity. Most mutants in this class also lack spectrally measurable cytochrome *b*; the only known exceptions are those containing mutations that affect the synthesis of the Rieske iron-sulfur protein and of cytochrome $c_1$ (30). Up to now, the following mutants have been identified: structural subunits (30, 90, 183), proteins involved in processing of the apocytochrome *b* pre-mRNA (37, 85, 108), proteins that promote translation of apocytochrome *b* transcripts (38, 145), posttranslational maturation of subunits (91), and proteins necessary for the assembly of the complex (201).

(iii) Mutants with mutations in the mitochondrial ATPase complex can be divided into two groups: those deficient in the $F_1$ ATPase and those deficient in the $F_0$ component. The hallmarks of $F_1$ mutants are the absence of ATPase activity and significantly lower concentrations of respiratory-chain components such as cytochrome oxidase and coenzyme $QH_2$-cytochrome *c* reductase. For this reason, $F_1$ mutants cannot be distinguished from the pleiotropic class by spectral criteria alone. Mutations blocking the synthesis of $F_1$ have no effect on the stability of mitochondrial DNA. Among the nuclear gene products known to affect $F_1$ are the structural subunits (151, 172, 173) and proteins that are necessary for translation or assembly of the subunits into oligomeric $F_1$ (S. Ackerman and A. Tzagoloff, unpublished results). $F_0$ mutants also exhibit a pleiotropic phenotype. They synthesize normal amounts of $F_1$, which is detected in mitochondria as an oligomycin-sensitive ATPase. In contrast to $F_1$ mutants, $F_0$ mutants are highly unstable and readily convert to *rho*$^-$ and *rho*$^0$ derivatives. The synthesis of a functional $F_0$ unit depends on the expression of nuclear genes that code for subunits of the complex (93, 132, 191) and on gene products that affect their assembly (Ackerman and Tzagoloff, unpublished). Since none of the mitochondrial $F_0$ genes (41) have introns, their expression does not depend on splicing factors. Conceivably, production of the mRNAs could require nuclear gene products involved in endonucleolytic processing of the primary transcripts. At present it is not known whether translation of this group of mitochondrial mRNAs is promoted by specific translation factors.

(iv) The phenotypic class of pleiotropic mutants is defined by strains lacking spectral cytochromes *b*, *a*, and $a_3$ but not cytochromes *c* or $c_1$. Pleiotropic mutants are generally defective in mitochondrial protein synthesis as a result of mutations in genes coding for ribosomal proteins, aminoacyl-tRNA synthetases, and translational initiation and

elongation factors. Nonconditional mitochondrial protein synthesis mutants exhibit a range of growth properties on nonfermentable substrates and convert to *rho*$^-$ and *rho*$^0$ derivatives at high frequency. The inability of a pleiotropic mutant to incorporate radioactive amino acid precursors into the mitochondrial translation products can occur for reasons other than a mutation in an essential component of the translational machinery. For example, fumarase mutants have an apparent mitochondrial protein synthesis-defective phenotype (200). This phenotype is probably due to a lowered intramitochondrial pool of aspartic acid in fumarase mutants.

(v) Mutants with normal levels of mitochondrial cytochromes and oligomycin-sensitive ATPase constitute the least extensively studied phenotypic class and probably represent a potpourri of different nuclear gene products. Those that have been identified include enzymes of the tricarboxylic acid cycle (105, 143, 174), of gluconeogenesis (155), and of the coenzyme Q (179) and lipoic acid (B. Repetto and A. Tzagoloff, unpublished observations) biosynthetic pathways.

## IDENTIFICATION OF MUTANTS FROM OTHER COLLECTIONS

The genes defined by some 20 complementation groups listed in Table 2 were identified by allelism tests with mutants characterized in other laboratories and by transformation with recombinant plasmids containing known *PET* genes. The pertinent references for these *PET* genes are provided in Table 2. Examples include (i) the *PET111* (formerly *E11*) and *PET494* genes that complement *pet* mutants in the collection of Ebner et al. (43, 44) (these genes have been shown to code for factors necessary for the translation of the mitochondrial mRNAs for subunits 2 and 3 of cytochrome oxidase, respectively [24, 43, 116, 169]); (ii) *CBS1* and *CBS2*, which complement mutations in the collection of Pillar et al. (135) and whose products are necessary for translation of the apocytochrome *b* mRNA (145); (iii) five subunits of coenzyme $QH_2$-cytochrome *c* reductase encoded by *RIP1*, *CYT1*, *COR2*, *COR4*, and *COR5* genes that were isolated either by a differential hybridization screening method coupled with hybrid-selected translation and immunoprecipitation (189, 190) or, in the case of *RIP1*, by use of a homologous *Neurospora crassa* probe (5); (iv) *CYC3* and *CYT2*, whose encoded ligases covalently couple heme to apocytochrome *c* (42, 148) and apocytochrome $c_1$ (A. Haid, personal communication), respectively; (v) *ATP4*, the gene for subunit 4 of the ATPase complex (191); (vi) *HEM2* and *HEM4*, coding for the δ-aminolevulinic acid dehydratase and coproporphyrinogen decarboxylase, respectively of the heme biosynthetic pathway (62); (vii) *OP1*, coding for the ATP-ADP exchange carrier protein (84, 126); (viii) *FBP1*, the gene for fructose-1,6-bisphosphatase (146, 155); and (ix) *TUFm*, the gene for mitochondrial elongation factor (121).

## *PET* GENES NOT MATCHED TO THIS MUTANT COLLECTION

Some 20 characterized *PET* genes have not yet been related to the complementation groups in Table 2. These genes (Table 3) are not all expected to be represented in the mutant collection. For example, *MRS1* codes for a protein that promotes excision of the bI3 intron from the long variant of apocytochrome *b* pre-mRNA (85, 135). This intron is missing in the mitochondrial genome of *S. cerevisiae* D273-10B/A1, the parental strain of the mutants in Table 2. Mutations in *MRS1* therefore cannot be expected to affect the growth of this strain on nonfermentable substrates. Mutations in *PET* genes coding for components of the yeast mitochondrial transcriptional machinery such as *RPO41*, because they promote a high rate of mitochondrial DNA loss (95, 96, 109), are also unlikely to be present among nonconditional *pet* mutants. This also applies to mutations in *VAS1* and *HTS1*, which code for the cytoplasmic and mitochondrial valyl- and histidyl-tRNA synthetases, respectively (16, 72, 123, 175).

Most of the genes listed in Table 3 are of the *PET* type. Some, however, have been included even though they do not meet the criteria of a *PET* gene in a strict sense. Mutations causing loss of catalytic activity of the histidyl- and valyl-tRNA synthetases encoded by *HTS1* and *VAS1* are lethal. Mutant alleles of both genes exist, however, that are altered only in the mitochondrial import signal sequence. These mutations block import of the synthetases into mitochondria and impart a respiratory-deficient phenotype, but they have no effect on the activity of the cytoplasmic enzymes (16, 123). Also on the borderline of *PET* classification are genes coding for enzymes in the heme biosynthetic pathway. Mutations in *HEM1* and *HEM13* express a heme requirement independent of the carbon source (62). At least two complementation groups (G32 and G88 in Table 2) in the *pet* collection consist of mutants with lesions in enzymes of heme biosynthesis, indicating that some mutants will exhibit differential growth properties on rich media containing fermentable versus nonfermentable substrates. We have therefore provisionally included *HEM1* and *HEM13* in the list of *PET* genes. There are also situations in which the growth phenotype of a *pet* mutant may change in response to the carbon source supplied in the medium. Porin mutants, for example, adapt reversibly to growth on glycerol following transfer from media containing glucose (110). A similar adaptation by strains harboring mutations in the 70-kilodalton (kDa) outer membrane protein has also been reported (144).

## NUCLEAR GENE PRODUCTS THAT ARE LOCATED IN MITOCHONDRIA AND DO NOT AFFECT RESPIRATION

Paradoxically, mutations in some components of the mitochondrial respiratory chain have no significant impact on the ability of *S. cerevisiae* to grow on nonfermentable substrates. Among such components are the similar iso-1- and iso-2-cytochrome *c* products of the *CYC1* and *CYC7* genes, respectively. Mutations in either gene alone fail to elicit a respiratory deficient phenotype because each protein is produced in sufficient quantity to support maximal electron transport. The absence of functional iso-1-cytochrome *c*, the major isolog, does, however, prevent growth of *S. cerevisiae* on lactate (164). Subunits 5a and 5b of cytochrome oxidase demonstrate another instance of two homologous mitochondrial proteins of which only one causes a *pet* phenotype when absent. In wild-type *S. cerevisiae*, subunit 5a is preferentially incorporated into the enzyme, which accounts for the lack of a phenotype in *cox5b* mutants (31, 32). The respiratory defect of *cox5a* mutants, however, can be complemented by the wild-type *COX5b* gene on a high-copy-number plasmid (31).

Some mitochondrial constituents, even though they may be subunits of respiratory enzymes, have no appreciable effect on electron transport. This is true of the 17-kDa

TABLE 3. *PET* genes not matched to this mutant collection

| Gene | Method of isolation[a] | Product | Reference(s) |
|---|---|---|---|
| *ATP5* | 2 | Oligomycin sensitivity-conferring protein | 93 |
| *BCY1* | 7 | Regulatory subunit of cyclic AMP-dependent kinase | 103, 176 |
| *COX9* | 1 | Subunit 7a of cytochrome oxidase | 198 |
| *CYP1* (*HAP1*) | 7 | Transcription factor for *CYC1* and *CYC7* | 20, 21, 29, 65, 192, 193 |
| *HAP2* | 7 | Nuclear transcription factor | 65, 136, 137 |
| *HAP4* | 7 | Nuclear transcription factor | 52 |
| *HEM1* | 7 | δ-Aminolevulinate synthase | 62, 185, 186 |
| *HEM13* | 7 | Coproporphyrinogen oxidase | 184, 205 |
| *HTS1* | 7 | Cytoplasmic and mitochondrial histidyl tRNA synthetase | 123, 175 |
| *LPD1* | 3 | Lipoamide dehydrogenase | 13, 35, 147, 149 |
| *MDH1* | 3 | Mitochondrial malate dehydrogenase | 105, 174 |
| *MIP1* | 7 | Catalytic subunit of mitochondrial DNA polymerase | 53, 58 |
| *MSS18* | 7 | COXI pre-mRNA splicing factor | 159 |
| *MRP7* | 3 | Mitochondrial ribosomal protein | 51 |
| *MRS1* | 7 | Cytochrome *b* bI3 intron splicing factor | 85, 86 |
| *NAM1* (*MTF2*) | 7 | Splicing of COXI pre-mRNA, translation | 7, 96 |
| *PIF1* | 7 | Mitochondrial DNA recombination factor | 54, 55 |
| *POR* | 6 | Porin | 110 |
| *RF1023* (*MTF1*) | 7 | Mitochondrial RNA transcription factor | 95 |
| *RPO41* | 3 | 145-kDa subunit of mitochondrial RNA polymerase | 76, 102 |
| *VAS1* | 3 | Cytoplasmic and mitochondrial valyl-tRNA synthetase | 16, 72 |
| *YMR31*[b] | 1 | Mitochondrial ribosomal protein | 104 |
| *YMR44*[b] | 1 | Mitochondrial ribosomal protein | 104 |
| – | 8 | Iron-sulfur protein of succinate dehydrogenase | 97 |
| –[b] | 3 | Lipoamide *S*-acetyl transferase | 124 |
| – | 7 | Transport, processing of coenzyme $QH_2$-cytochrome *c* reductase subunits | 91 |
| – | 1 | 70-kDa outer membrane protein | 69, 144 |
| – | – | Intermembrane space protease | 141 |

[a] 1, Plasmid or bacteriophage library screen with a synthetic DNA probe based on protein sequence data; 2, plasmid library screen by differential hybridization to polysomal mRNA associated with mitochondria versus an excess of mRNA from non-organelle-bound ribosomes, followed by in vitro translation of hybrid-selected mRNA and immunoprecipitation; 3, λgt11 library screen with either monoclonal or polyclonal antibodies; 4, plasmid library screen with poly(A)$^+$ size-selected mRNA versus an excess of mRNA from glucose-repressed respiratory deficient cells, followed by in vitro translation of hybrid-selected mRNA and immunoprecipitation; 5, λ or plasmid library screen with a probe from a gene with sequence similarity; 6, cDNA library screen for inserts followed by in vitro translation of hybrid-selected mRNA and immunoprecipitation; 7, transformation of mutant with genomic library; 8, polymerase chain reaction synthesis of DNA with primers based on partial protein sequence.

[b] The phenotype of mutants with mutations in these genes has not been reported.

subunit of coenzyme $QH_2$-cytochrome *c* reductase encoded by *COR3* (30, 188) and the cytochrome oxidase subunit 8 encoded by *COX8* (131). Also dispensable with respect to respiration are two tRNA modification enzymes, $\Delta^2$-isopentenyl pyrophosphate transferase, encoded by *MOD5* (39, 92, 122), and guanosine $N^2$,$N^2$-dimethyltransferase, encoded by *TRM1*; the mitochondrial ribosomal protein encoded by *MRP13* (130); a 45-kDa protein of the outer membrane (202); the nonspecific nuclease product of *NUC1* (194); and enzymes such as manganous superoxide dismutase (101, 190) and cytochrome *c* peroxidase (63, 75). The last two enzymes provide protection against the persistence of destructive radicals. It is of interest that mutations in the mitochondrial alcohol dehydrogenase (*ADH3*), tetrahydrofolate synthase (*MIS1*), and citrate synthase (*CIT1*) genes are not deleterious to respiration (160, 171, 204). The absence of these activities in mitochondria must therefore be compensable by the cytoplasmic isoenzymes. Mutations in three of the gene products in Table 4 affect the ability of *S. cerevisiae* to metabolize only a specific nonfermentable substrate. One already mentioned is iso-1-cytochrome *c* (112, 167). The second, cytochrome $b_2$, is mitochondrial lactate dehydrogenase (66, 67); mutations in the structural gene for this protein therefore prevent utilization of lactate as a substrate. Additionally, of the two yeast citrate synthases, only the mitochondrial enzyme encoded by *CIT1* (171) is necessary for growth on acetate (77).

Even though this review is meant to catalog and cross-reference yeast nuclear genes necessary for respiration, it is hard to ignore the role of mitochondria in compartmentalizing different metabolic pathways that do not bear on the respiratory potential of the cell. Examples of genes for this class of mitochondrial constituents are currently confined to those encoding a few enzymes in amino acid biosynthetic and utilization pathways. *ILV2* (48, 49, 100, 138) and *ILV5* (74, 133, 138) encode the first two enzymes in the isoleucine-valine biosynthetic pathway, acetohydroxy acid synthase and acetohydroxy acid reductase, respectively. *LEU4* (3, 6, 15) encodes the first enzyme, α-isopropylmalate synthase, in the biosynthetic pathway committed to leucine production. *PUT1* (12, 195, 196) and *PUT2* (11, 12, 87) code for the two enzymes in the proline utilization pathway. Localization of proline oxidase and $\Delta^1$-pyrroline-5-carboxylate dehydrogenase to the mitochondrion separates these catabolic enzymes from the proline biosynthetic pathway in the cytoplasm.

Finally, we mention still another class of genes coding for proteins that function in the transport of cytoplasmically synthesized proteins into mitochondria. Since mutations in these genes are lethal they cannot be considered *PET* genes. At present, examples of such genes are *MAS1* and *MAS2*, whose products process mitochondrial target sequences (139, 197) and *HSP60* and *SSC1*, which code for proteins involved in the assembly of mitochondrial polypeptides into functional complexes (17, 27, 28, 142).

TABLE 4. Genes that are not *PET* but code for mitochondrial constituents

| Gene | Method of isolation[a] | Product | Reference(s) |
|---|---|---|---|
| *ADH3* | 5 | Mitochondrial alcohol dehydrogenase | 204 |
| *CCP* | | Cytochrome *c* peroxidase | 63, 75 |
| *CIT1* | 2 | Mitochondrial citrate synthase | 171 |
| *COR3* | 4 | 17-kDa subunit of coenzyme $QH_2$-cytochrome *c* reductase | 188, 189 |
| *COX5b* | 7 | Subunit 5b of cytochrome oxidase | 31, 32 |
| *COX8* | 1 | Subunit 8 of cytochrome oxidase | 131 |
| *CYC1* | 1 | Iso-1-cytochrome *c* | 112, 167 |
| *CYC7* | 5 | Iso-2-cytochrome *c* | 113 |
| *HSP60* | 3, 7 | Heat shock protein HSP60 | 17, 142 |
| *ILV2* | 7 | Acetohydroxy acid synthase | 48, 49, 100, 138 |
| *ILV5* | 7 | Acetohydroxy acid reductoisomerase | 74, 133, 138 |
| *LEU4* | 7 | α-Isopropyl malate synthase | 3, 6, 15 |
| *MAS1* (*MIF1*, *PEP*) | 7 | Transit sequence protease enhancer | 197, 203 |
| *MAS2* (*MIF2*, *MPP*) | 7 | Transit sequence protease | 139, 203 |
| *MIS1* | 1 | Tetrahydrofolate synthase | 160 |
| *MOD5* | 7 | $\Delta^2$-Isopentenyl pyrophosphate transferase | 39, 92, 122 |
| *MRP13* | 3 | Mitochondrial ribosomal protein | 130 |
| *NUC1* | 3 | Mitochondrial nuclease | 194 |
| *OM45* | 1 | 45-kDa outer membrane protein | 202 |
| *PUT1* | 7 | Proline oxidase | 12, 195, 196 |
| *PUT2* | 7 | $\Delta^1$-Pyrroline-5-carboxylate dehydrogenase | 11, 12, 87 |
| *SOD* | 4 | Manganous superoxide dismutase | 101, 190 |
| *SSC1* | 5 | Heat shock protein HSP70 | 27, 28 |
| *TRM1* | 7 | Guanosine $N^2$,$N^2$-dimethyltransferase | 45, 46, 134 |
| – | 3 | Cytochrome $b_2$ | 66, 67 |

[a] See Table 3, footnote *a*, for key.

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