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The Mitochondrial Genotype Can Influence Nuclear Gene Expression in Yeast

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Isochromosomal, respiratory-deficient yeast strains, such as a mit$^-$, a hypersuppressive petite, and a petite lacking mitochondrial DNA, are phenotypically identical in spite of differences in their mitochondrial genomes. Subtractive hybridizations of complementary DNA's to polyadenylated RNA isolated from derepressed cultures of these strains reveal the presence of nuclear-encoded transcripts whose abundance varies not only between them and their respiratory-competent parent, but among the respiratory-deficient strains themselves. Transcripts of some nuclear-encoded mitochondrial proteins, like cytochrome c and the α and β subunits of the mitochondrial adenosine triphosphatase, whose abundance is affected by glucose or heme, do not vary. In the absence of major metabolic variables, yeast cells seem to respond to the quality and quantity of mitochondrial DNA and modulate the levels of nuclear-encoded RNA's, perhaps as a means of intergenomic regulation.

THE SYNTHESIS OF A NUMBER OF mitochondrial proteins in *Saccharomyces cerevisiae* can be affected by the levels of glucose (catabolite repression), oxygen, and heme (*1*). These effects are most evident by large changes in the cell's respiratory capacity that accompany these "metabolic" variables. Recent studies have focused on the molecular basis for regulation of a few mitochondrial proteins encoded in the nucleus, like cytochrome c (the product of the *CYC1* gene) (*2*), the α and β subunits of the mitochondrial F1 adenosine triphosphatase (F1 ATPase) (*3*), and some subunits of cytochrome oxidase (*4, 5*). The most detailed molecular information available on the regulation of expression of these genes is that for *CYC1*: control of cytochrome c synthesis is largely transcriptional and involves cis-acting upstream activation sites (UAS) that are analogous in some ways to higher eukaryotic enhancers (*2*); these sites are believed to control *CYC1* expression in response to glucose and heme levels through interactions with trans-acting transcriptional factors (*6*).

Since the biogenesis of functional mitochondria requires a large contribution from the nucleus, many nuclear genes might be regulated, and some, perhaps, independent of the metabolic variables noted above. To examine these questions, we have used complementary DNA (cDNA) subtractions to detect differentially expressed transcripts among isonuclear, derepressed yeast strains that differ only by the presence or absence of the kind of mitochondrial DNA (mtDNA) they contain. We show that some nuclear DNA sequences are differentially expressed between the respiratory competent cells (ρ^+) and isonuclear, respiratory-deficient derivatives, and others are differentially expressed among the respiratory-deficient cells themselves. These results suggest a hitherto undescribed type of nuclear-mitochondrial interaction whereby nuclear DNA sequences can respond to the state of the mitochondrial genome.

Experiments were carried out with four isonuclear yeast (*Saccharomyces cerevisiae*) strains all grown on raffinose, a fermentable but nonrepressing sugar. Three of the strains are respiratory-deficient with different lesions in their mitochondrial genomes; all are derived from COP161, a respiratory-competent ρ^+. One strain is a mit$^-$ (E69) containing a deletion in the *oxi2* gene (*7*); another strain is a hypersuppressive cytoplasmic petite (ρ^-), designated HS40, in which all but about 700 base pairs (bp) of the wild-type mitochondrial genome are deleted; the other strain is a ρ^0 petite that completely lacks mtDNA. Although mit$^-$ E69 is unable to respire because it lacks subunit III of cytochrome oxidase, it can still transcribe and translate its stable mitochondrial genome. The mitochondrial genome of HS40 ρ^- contains an *ori/rep* sequence, one of several closely related sequences believed to function as preferred origins of mtDNA replication (*8*); like other ρ^- petites, HS40 lacks mitochondrial protein synthesis, but it can transcribe sequences on its truncated genome. Thus, although these strains are phenotypically identical with respect to respiratory function, they contain different mtDNA lesions that give rise to the same respiratory-deficient phenotype.

To determine whether the steady-state levels of any cytoplasmic RNA's might be differentially regulated in these respiratory-deficient cells, cDNA subtractions were carried out between mit$^-$ E69 and the ρ^0 petite, and between the HS40ρ^- and ρ^0 petites. The former pair represents, in principle, the largest difference between cells with defective mitochondrial genomes; the latter pair represents a more stringent test for transcripts that might be differentially regulated among respiratory-deficient cells since both cell types are genotypically petite. In each case, single-stranded cDNA's were prepared by reverse transcription of polyadenylated [poly(A)$^+$] RNA fractions of cytoplasmic RNA isolated from cultures in the mid-logarithmic phase of growth, and hybridized with the heterologous poly(A)$^+$ RNA fraction to a $R_o t$ of about 4000. Under these conditions the cDNA's of low abundance transcripts that are not differentially expressed should be quantitatively driven into hybrid. The single-stranded cDNA's were isolated by hydroxyapatite chromatography and cloned into the Pst I site of plasmid pBR322 after second-strand synthesis and GC-tailing (*9*).

The cloned cDNA inserts were initially screened by differential colony hybridization with ^{32}P-labeled single-stranded cDNA probes; these were prepared by reverse transcription of poly(A)$^+$ RNA fractions isolated from the ρ^+, and each of the respiratory-deficient strains. Plasmid DNA's isolated from positive clones were then screened by dot-blot hybridization with the same single-stranded cDNA probes. False positives and cDNA's derived from mtDNA were screened out, and selected cDNA clones were analyzed by Northern blotting of poly(A)$^+$ RNA. The input RNA's were normalized to the amount of actin message present in each preparation. Actin, which is an essential product in yeast required for

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many cellular functions (*10*), is a suitable standard for this purpose since the amount of actin messenger RNA (mRNA) is the same in both repressed and derepressed cells (*3*) and in all four of the strains used in our study. At the same time, the various probes were analyzed by Southern blotting to assess the genomic organization of potentially regulated sequences. Finally, the cDNA's were subcloned into M13 vectors and all of the inserts were sequenced either wholly or in part.

From the Northern analysis of selected members of cDNA clones, we have identified two classes of nuclear-encoded transcripts, the amount of each varying according to the kind of mtDNA in the cell (Fig. 1). The expression of class I RNA's correlates only to the respiratory capacity of the cell. The abundance of these transcripts is increased relative to the ρ^+, and to about the same extent in all of the respiratory-deficient strains. It is not immediately obvious why cDNA's to these class I RNA's were selected since the cDNA subtractions were carried out between the respiratory-deficient strains. One possibility is that they were simply fortuitously identified because of extensive screening and because the cDNA subtraction procedure was not completely stringent. In any case, we present two examples of class I RNA's that are detected with the cDNA probes mit30 (0.3 kb) and mit78 (0.2 kb), both obtained from the mit$^-$ E69 by ρ^0 subtraction. The mit30 and mit78 cDNA's are derived from small transcripts (0.5 kb and 0.45 kb, respectively) whose increased abundance correlates only with the absence of respiration rather than with the kind of lesion of the mitochondrial genome. Both mit30 and mit78 cDNA's, which hybridize to different Eco RI and Hind III genomic DNA fragments (Fig. 1), contain an open reading frame over the entire length of the available DNA sequences. Mit30, moreover, contains two open reading frames, one in each complementary strand. Whether these transcripts respond similarly to any respiratory defect, mitochondrial or nuclear, remains to be determined. Except for short pyrimidine-rich stretches, their DNA sequences (Fig. 2), or deduced amino acid sequences, show no homology to each other or to any sequences in the MicroGenie Data Bank.

Class II RNA's are petite-specific since they are more abundant in the petites than in either mit$^-$ E69 or the ρ^+. Moreover, some of the class II transcripts are significantly more abundant in one petite or the other, although the magnitude of difference can vary from experiment to experiment. For example, the cDNA ρ19 (0.2 kb) hybridizes to multiple RNA species that are most abundant in the ρ^0 petite (Fig. 1). These transcripts range in size from about 1.5 to 0.5 kb, and are barely detected in total or poly(A)$^+$ RNA fractions from ρ^+ cells. The broad distribution RNA's detected with

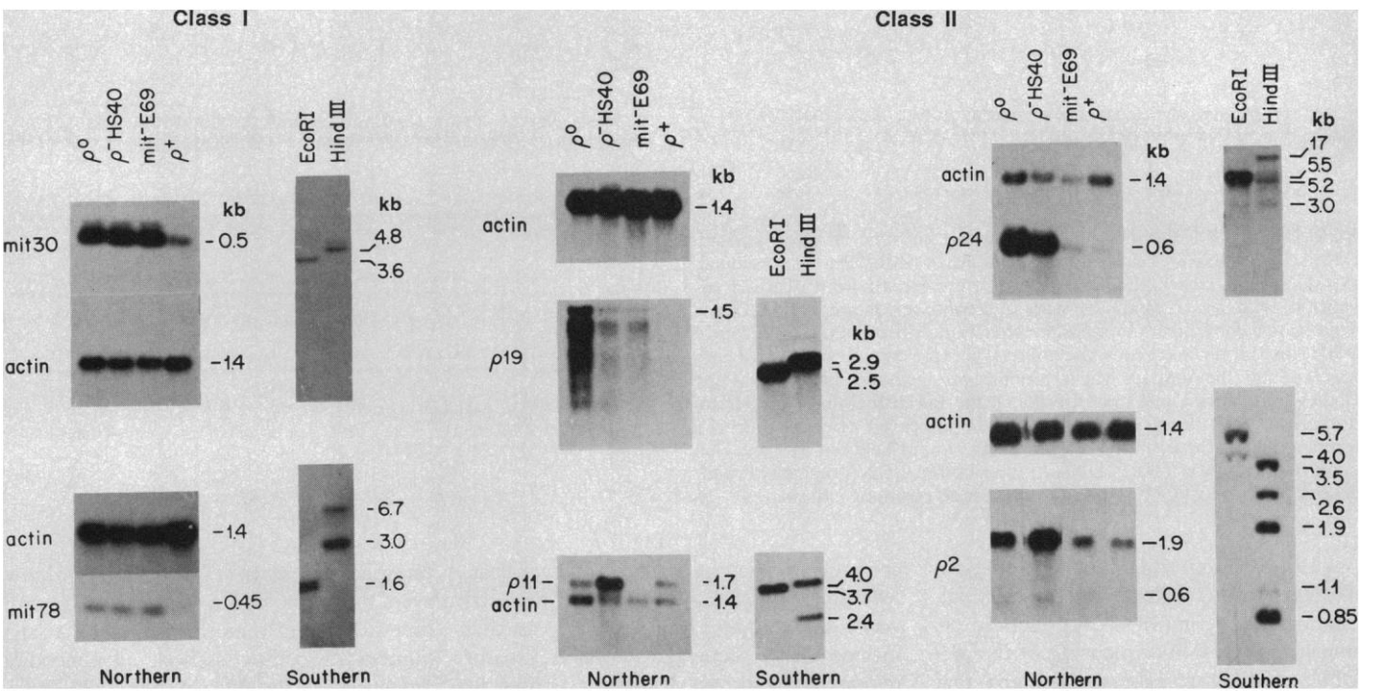

Fig. 1. Northern and Southern blot analysis of selected cDNA clones. Northern blots: The three isonuclear, respiratory incompetent strains ρ^0, HS40 ρ^-, and mit$^-$ E69 were derived from the respiratory competent COP161 ρ^+ (a Ade Lys) strain. Total RNA was prepared from cells in the mid-log phase of growth on liquid medium [1 percent (w/v) yeast extract, 1 percent (w/v) Bactopeptone, and 2 percent (w/v) raffinose] by the method of Chirgwin *et al.* (*28*), or by modification of the small-scale procedure of Nasmyth (*29*). Poly(A)$^+$ RNA fractions were prepared by two passages of total RNA through an oligo(dT) cellulose column (*30*) or by selective binding and elution on Hybrid-mAP paper (Amersham). Poly(A)$^+$ RNA was fractionated by electrophoresis in 1.25 percent agarose gels containing 5 m*M* methyl mercury hydroxide. After electrophoresis, the RNA was transferred to a zeta-probe membrane and hybridized with the indicated ^{32}P-labeled cDNA probe. Hybridizations were carried out for 16 to 18 hours at 42°C in 50 percent formamide, 744 m*M* NaCl, 50 m*M* NaH_2PO_4, 5 m*M* EDTA (*p*H 7.4), 0.1 percent (w/v) each of bovine serum albumin, Ficoll, polyvinylpyrollidone, 1 percent SDS, and sonicated calf thymus DNA (100 μg/ml). After hybridization, the blots were washed once for 30 minutes at 65°C in 300 m*M* NaCl, 30 m*M* sodium citrate, and 0.5 percent SDS and then twice for 30 minutes at 65°C in 150 m*M* NaCl, 15 m*M* sodium citrate, and 0.5 percent SDS. Levels of RNA were normalized to the signal obtained with actin DNA derived from the actin plasmid pYact1, which contains the entire yeast actin gene (*31*). In some cases, blots were probed again with the pYact1 probe after removal of the original probe by washing the blot three times in boiling 15 m*M* NaCl, 1.5 m*M* sodium citrate and 0.1 percent SDS and then rinsing at 25°C in the same medium. Southern blots: Total yeast DNA, isolated from the ρ^0 strain (*32*), was digested with Eco RI or Hind III and fractionated on an 0.8 percent agarose gel. The DNA was then transferred to a nitrocellulose membrane and hybridized with the same cDNA probes as for the Northern blots at 65°C in 744 m*M* NaCl, 50 m*M* NaH_2PO_4, 5 m*M* EDTA (*p*H 7.4), 0.1 percent (w/v) each of bovine serum albumin, Ficoll, polyvinylpyrollidone, 0.2 percent SDS, and sonicated calf thymus (200 μg/ml) DNA. Blots were washed as described above for Northern blots. A Hind III digest of λ DNA was used for size standards. I and II refer to class I and class II transcripts, respectively.

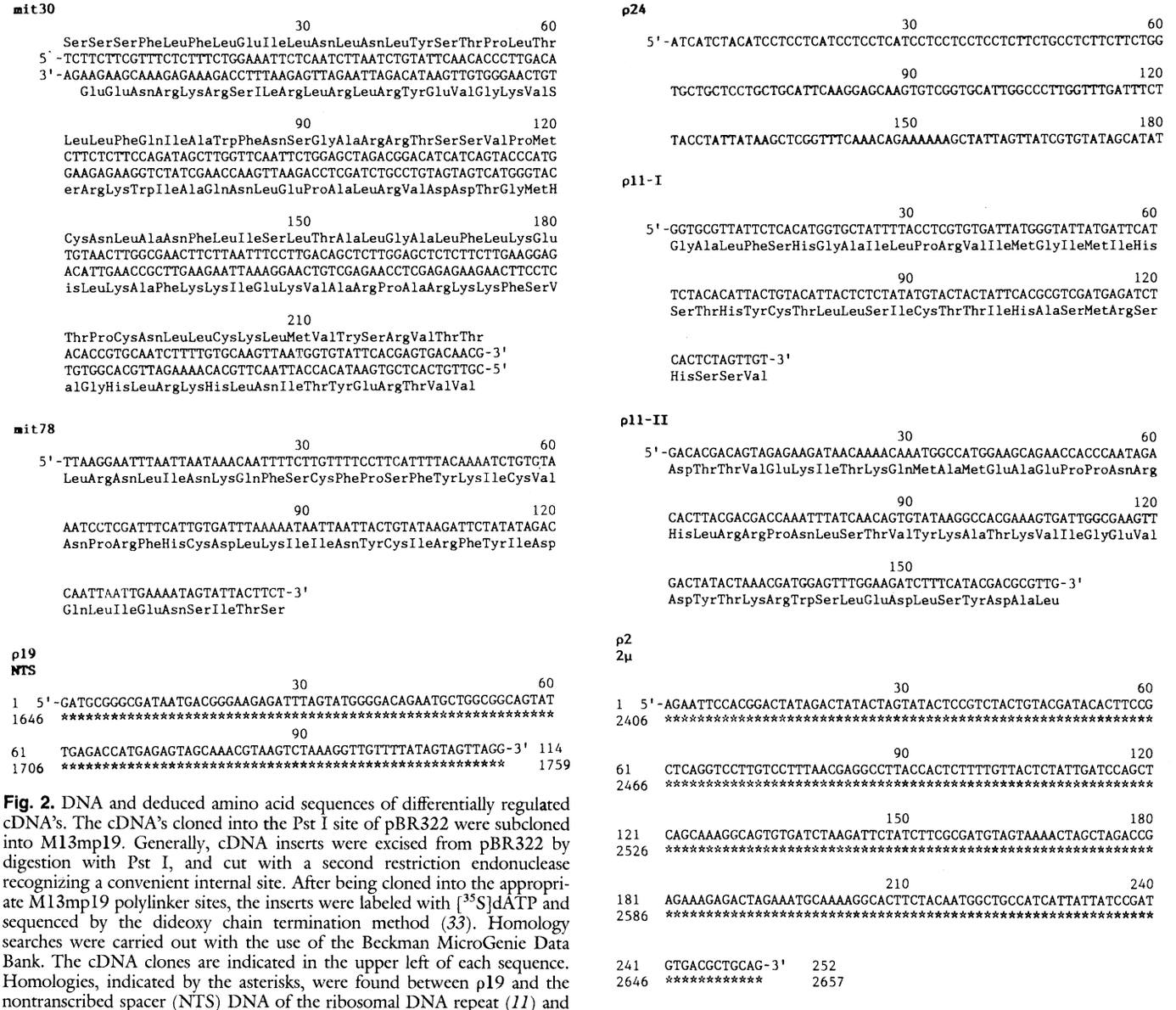

Fig. 2. DNA and deduced amino acid sequences of differentially regulated cDNA's. The cDNA's cloned into the Pst I site of pBR322 were subcloned into M13mp19. Generally, cDNA inserts were excised from pBR322 by digestion with Pst I, and cut with a second restriction endonuclease recognizing a convenient internal site. After being cloned into the appropriate M13mp19 polylinker sites, the inserts were labeled with [^{35}S]dATP and sequenced by the dideoxy chain termination method (*33*). Homology searches were carried out with the use of the Beckman MicroGenie Data Bank. The cDNA clones are indicated in the upper left of each sequence. Homologies, indicated by the asterisks, were found between ρ19 and the nontranscribed spacer (NTS) DNA of the ribosomal DNA repeat (*11*) and between ρ2 and 2μ DNA (*17*) at the nucleotide positions indicated.

the ρ19 probe cannot be be due to nonspecific degradation of the RNA since essentially undegraded actin mRNA is present in the same samples. DNA sequencing of the ρ19 cDNA (Fig. 2) reveals, surprisingly, that these transcripts are derived from the spacer DNA region of the chromosomal ribosomal DNA (rDNA) repeat located between the 5*S* rRNA gene and the start of transcription of the 35*S* rRNA precursor (*11*); a portion of that sequence and its location within the rDNA repeat is shown in Fig. 2. The transcripts detected with the ρ19 probe are derived from the same strand as the 35*S* rRNA precursor (*12*), which is opposite to the template strand for 5*S* rRNA transcription (*13*). Additional mapping will be required to delimit the boundaries of these unusual transcripts.

Although the extent to which rDNA spacer sequences may be transcribed is unclear, Swanson and Holland (*14*) have reported very low abundance transcripts in yeast that include these sequences. Possibly, spacer regions are transcribed, but the transcripts turn over rapidly in ρ^+ cells so that they are not readily detected in measurements of steady-state levels of RNA. Such transcripts, however, may be more stable in a ρ^0 petite.

The ρ24 fragment (0.45 kb) is an example of a cDNA that hybridizes to a 0.6-kb transcript preferentially expressed in the hypersuppressive and ρ^0 petites (Fig. 1). This cDNA hybridizes to multiple Hind III and Eco RI genomic DNA fragments, although most prominently to a 5.5-kb Eco RI fragment (Fig. 1). A portion of ρ24 has been sequenced, and this sequence shows some homology to the 5′ nontranslated region of the yeast *TRP3* and *P6* (repressible acid phosphatase) genes (*15, 16*) (Fig. 2). However, the homologous regions are pyrimidine-rich tracts that are found in a large number of coding as well as noncoding sequences in the MicroGenie Data Bank. Therefore, we cannot at present make any conclusions about the identity of ρ24 transcripts.

Two other examples of petite-specific cDNA's, ρ11 and ρ2 (Fig. 1), hybridize to RNA's most abundant in the hypersuppressive petite; ρ11 is a 1.6-kb cDNA that hybridizes to a single 1.7-kb transcript (Fig. 1). We have sequenced portions of two nonoverlapping fragments of ρ11 (a 0.3-kb Hpa II fragment and a terminal 0.7-kb Pst I–Hind III fragment), and both fragments contain an open reading frame over their entire length (Fig. 2). We have not found any homology between ρ11 DNA or its

deduced amino acid sequences to any other known sequences.

The petite specific ρ2 cDNA (0.45 kb) hybridizes to a 1.9-kb transcript and to a minor 0.6-kb species, which are most abundant in HS40 ρ^-, and to multiple Hind III and Eco RI fragments of genomic DNA (Fig. 1). The DNA sequence of ρ2 (Fig. 2) shows that it is derived from the D region of the yeast 2μ plasmid (*17*). This region is included within 1.95- and 0.7-kb transcripts of 2μ DNA (*18*), consistent with our Northern analysis. To determine whether other 2μ transcripts show a similar pattern of abundance, Northern blots of poly(A)$^+$ RNA fractions were probed with a 6-kb variant of the 2μ plasmid (*19*). These data (Fig. 3) show that the major 2μ transcripts (*20*) are also most abundant in HS40 ρ^-. This increased abundance is partly explained by a twofold increase in the copy number of 2μ plasmid DNA in HS40 ρ^- (*12*); however, the major 2μ transcripts are 10 to 15 times more abundant in HS40 ρ^- than in the other strains. Since 2μ copy number appears to be linked to its expression (*18*), it would not be surprising to find a relation between transcript abundance and plasmid copy number.

Having established a relation between the abundance of some cytoplasmic RNA's and the kind of mtDNA in the cell, we were also interested in determining whether transcripts to any known nuclear-encoded mitochondrial proteins would respond similarly. Accordingly, Northern blot analyses were carried out on the wild-type and the respiratory-deficient strains with probes for the genes encoding the α and β subunits of the mitochondrial F_1 ATPase, cytochrome c, and subunit VI of cytochrome oxidase. These genes are of particular interest in the context of our studies since their transcription is known to be sensitive to catabolite repression (*3, 4, 5, 21*), and for *CYC1*, and perhaps cytochrome oxidase VI, to the levels of heme (*2*).

Although the mRNA's for these nuclear-encoded mitochondrial proteins are increased at least five to ten times when cells are derepressed (*3, 4, 5, 21*), only transcripts for cytochrome oxidase subunit VI show a significant differential response among the respiratory-deficient strains (Fig. 4). Surprisingly, a 1.4-kb transcript detected with the subunit VI probes is more abundant in the petites than in the mit$^-$ E69 or ρ^+ cells. The hybridization probes that we used to detect these transcripts (two Bgl II fragments of 1.3 and 1.5 kb) were derived from the plasmid YEpVI-3 (*4, 5*), which contains a 4.3-kb yeast DNA insert that includes the entire subunit VI coding region and flanking sequences. Using the same Bgl II

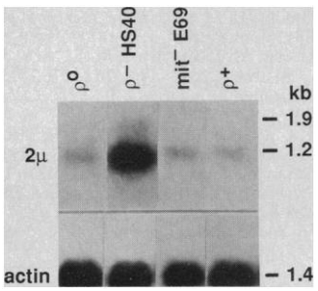

Fig. 3. Northern blot analysis of 2μ transcripts. Poly(A)$^+$ RNA's were probed with α-^{32}P-labeled 6-kb Eco RI fragment of cloned 2μ DNA (T2μ-1). RNA isolation and Northern blot analysis were as described in Fig. 1.

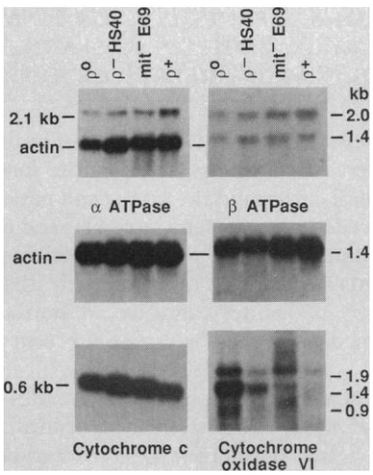

Fig. 4. Northern analysis of nuclear genes encoding known mitochondrial proteins. Northern blot hybridizations to poly(A)$^+$ RNA's were as described in Fig. 1. The probes were the α and β subunits of ATPase [pBR12-5, α subunit, and pVu 322-β2, β subunit (*4, 34*)], a 0.6-kb Eco RI–Hind III fragment containing the *CYC1* gene cloned into pBR322, and Bgl II fragments 4 and 5 from the YEpVI-3 plasmid containing the subunit VI gene (*4, 5*).

probes, Wright *et al.* (*4*), also observed multiple poly(A)$^+$ RNA's in Northern blots, but these were not characterized with respect to their origins and termini. Although it is not clear why among these genes only transcripts of cytochrome oxidase subunit VI vary, and particularly, why they appear more abundant in petites than in the ρ^+, the results nevertheless allow a clear distinction between metabolic regulation through catabolite repression or heme levels, and the differential expression of transcripts described here.

There are indications that a number of cellular processes can be affected by the state of the mitochondrial genome, independent of obvious metabolic variables. For example, the ability of yeast cells to utilize certain sugars varies in different petites (*22*), suggesting that the expression of some pathways of sugar utilization can in some way be influenced by different, defective mitochondrial genomes. While the molecular basis for this phenomenon is unclear, it does bear some similarity to our observations. Another related observation is that a linear, double-stranded DNA killer plasmid from *Kluyveromyces lactis*, when introduced into *Saccharomyces cerevisiae*, is unstable in both ρ^+ and mit$^-$ cells, but is stable in ρ^0 petites (*23*).

Although the relation of the above (partially characterized) transcripts to mitochondrial functions remains to be established, as does their mode of regulation, nevertheless the abundance of these RNA's is increased in one or more of the respiratory-deficient strains, as if these cells are attempting to compensate for their respiratory-deficient defect or for the particular mtDNA lesion that they harbor. Cellular attempts at copy number control, which are poorly understood for yeast mtDNA, might be manifest in such a way.

How might expression of some nuclear genes be affected differently in these respiratory-deficient cells? One possibility is that a mitochondrial gene product is exported and functions outside the organelle, for example, as a negative regulatory element. According to this model, control of expression of some nuclear genes would be maintained as long as cells contained a functional mitochondrial genome. Upon conversion to petites, however, the cells could no longer synthesize the putative regulator since petites have lost the ability to carry out mitochondrial protein synthesis, and they lack most, and in some cases all, of the mitochondrial transcripts found in ρ^+ or mit$^-$ cells. Although protein or RNA export from mitochondria has not been demonstrated directly, there are some indications that such export may occur. These come primarily from the observations that a particular murine cell surface antigen, although specified in part by a gene in the major histocompatibility complex, is maternally inherited (*24*), and that expression of this antigen is affected in cell fusion experiments by rhodamine 6G (*25*), a reagent specific for mitochondria.

The notion of partitioning of regulatory molecules between the mitochondria and the nucleus is not restricted, of course, to products of the mitochondrial genome. There is now good evidence for the partitioning of some nuclear gene products between the mitochondria and extramitochondrial compartments (*26, 27*). Thus, nuclear-encoded regulatory factors could partition between the mitochondria and the nucleus, and their relative distributions could be determined by the presence or absence, or the amounts, of mtDNA (or RNA) sequences. Thus a mechanism would exist for regulation of nuclear gene expression in response

to the quality as well as the quantity of mtDNA or RNA sequences. In that way, the nuclear genome could "sense" the amount or kind of mtDNA in the cell.

Whether different ρ^- mtDNA's, such as those containing the genes for the large or small rRNA's, both of which can be transcribed and correctly processed in petites, can affect expression of genes encoding mitochondrial ribosomal proteins or other nuclear-encoded mitochondrial translation factors remains to be determined. Many similar questions can also be asked concerning other sequences along the yeast mitochondrial genome.

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Human Neuroelectric Patterns Predict Performance Accuracy

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In seven right-handed adults, the brain electrical patterns before accurate performance differed from the patterns before inaccurate performance. Activity overlying the left frontal cortex and the motor and parietal cortices contralateral to the performing hand preceded accurate left- or right-hand performance. Additional strong activity overlying midline motor and premotor cortices preceded left-hand performance. These measurements suggest that brief, spatially distributed neural activity patterns, or "preparatory sets," in distinct cognitive, somesthetic-motor, and integrative motor areas of the human brain may be essential precursors of accurate visuomotor performance.

PREPARATORY SET FOR HUMAN VISUOMOTOR performance, defined as a state of readiness to receive a stimulus or make a response (*1*), has been studied by a variety of disciplines. Temporal properties of preparatory sets have been measured in information-processing studies, but such studies have not focused on the underlying neural systems (*2*). Spatial properties of preparatory sets measured in cerebral blood flow studies have revealed increased metabolic activity for sensory-specific focus of attention in superior prefrontal, midfrontal, and anterior parietal cortices (*3*). These studies have been limited, however, by the temporal resolution (1 minute or longer) of blood flow measurement techniques. Clinical neuropsychological studies have demonstrated that behaviors requiring preparatory sets (*4*) rely on intact lateral frontal regions (*5*), but variability in size and location of lesions has limited the spatial specificity of such studies in localizing normal function. And although scalp-recorded brain electrical and magnetic recordings provide both spatial and temporal information on neural activity underlying preparatory sets, studies of the contingent negative variation (CNV), an event-related brain potential component thought to be related to preparatory set, have often yielded controversial or ambiguous results (*6*).

Recording from 26 electrodes and using several signal-enhancing procedures, we measured the rapidly changing spatial patterns of mass neuroelectric activity associated with preparation and execution of precise right- and left-hand finger pressures in response to visual numeric stimuli. We found differences occurring during the prestimulus period between patterns associated with subsequently accurate and inaccurate performance. These group differences allowed discrimination of subsequent performance accuracy for both hands of individual subjects. Thus, a spatially specific, multicomponent neural preparatory set, composed of an invariant left frontal component and hand-specific central and parietal components, may be essential for accurate performance of certain types of difficult visuomotor tasks.

Seven healthy, right-handed male adults were recruited from the community and paid for their participation. They were required to exert rapid, precisely graded pressures (forces from 0.1 to 0.9 kg) followed by immediate release, with right- and left-hand index fingers in response to visual numeric stimuli (numbers 1 to 9). The stimulus was presented randomly on successive trials 1 second after a cue (the letter *V* lasting 0.3 second) that was slanted at a 30° angle to the right or left to indicate the required response hand (*7*). In "respond" trials, the

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