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A Path from Mitochondria to the Yeast Nucleus

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A path from mitochondria to the yeast nucleus

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We have identified a path in yeast, from mitochondria to the nucleus, which may have a regulatory function in mitochondrial biogenesis. This path is evident as an elevated expression of a number of nuclear DNA sequences in response to specific defects in the mitochondrial genome, including the absence of mitochondrial DNA in ρ^0 petites. Among those nuclear sequences preferentially expressed in certain respiratory-deficient cells are stable poly(A)$^+$ transcripts derived from the so-called non-transcribed spacer region of the nuclear ribosomal DNA repeat, where they are most abundant in the ρ^0 petite. Although the function of these unusual RNAs is unclear, the observations may reflect the presence of a mitochondrial homeostatic control system in yeast, which we suggest could function to adjust the mass of mitochondria and mitochondrial DNA in the cell in response to inequities in organelle apportionment during cell budding.

Introduction

There are now many examples of the various contributions made by the nuclear genome to mitochondrial biogenesis. Most of the proteins required for mitochondrial function and the operation of its genetic apparatus are encoded in the nucleus, synthesized in the cytoplasm and imported into the organelle. The details of the import and assembly processes, and the specific nuclear-gene product requirements for mitochondrial gene expression are now being clarified at the molecular level (Tzagoloff & Myers 1986). Recent evidence suggests, moreover, that the nuclear contribution to mitochondrial biogenesis is not restricted to proteins, but may even include nucleic acids (Chang & Clayton 1987). Superimposed on this nucleus-to-mitochondria flow of materials is the regulation of synthesis of nuclear-encoded components of the oxidative phosphorylation apparatus, especially evident in yeast in catabolite repression and haem control. The molecular mechanisms underlying these controls are known to involve *cis*- and *trans*-acting regulatory elements (Guarente & Hoar 1984; Pinkham & Guarente 1985; Pfeifer *et al.* 1987*a*, *b*).

But what of signals from mitochondria to the nucleus? Can the nuclear genome detect and respond to differences in mitochondria, independent of major metabolic differences between respiratory-deficient and respiratory-competent cells? This paper summarizes recent evidence (Parikh *et al.* 1987) that the levels of expression of some nuclear DNA sequences in yeast correlate with the quality or quantity of mitochondrial DNA (mtDNA); this evidence suggests that there is a path of communication from mitochondria to the nucleus. Evidence is also summarized which suggests that amplification of mtDNA, and probably of mitochondrial mass, occurs during diploid bud formation. These two seemingly unrelated observations are brought together by a hypothesis that we present for a control circuit between mitochondria and the nucleus, designed so that budding yeast cells can monitor and respond to mitochondrial apportionment during growth and differentiation.

Differential expression of nuclear DNA sequences in respiratory-deficient cells

We reasoned that, if there is a path of communication from mitochondria to the nucleus whose function is to control some aspect of mitochondrial biogenesis, likely signals in that path might be the functional state or the quantity of mtDNA. To examine these possibilities, we have used complementary DNA (cDNA) subtraction analysis to determine if any transcripts derived from the nuclear genome are differentially expressed in a set of isochromosomal respiratory-deficient cells that differ in the quality or quantity of mtDNA. To minimize metabolic variables, all cells were grown on raffinose, a fermentable but non-repressing sugar. These respiratory-deficient strains were derived from the same parental ρ^+ and included a mit$^-$ (E69), harbouring a deletion in the *oxi*2 gene (encoding cytochrome oxidase subunit III), a ρ^- petite (HS40), which has retained about 700 base pairs (bp) of the wild-type mitochondrial genome (including a putative origin of DNA replication), and a ρ^0 petite lacking mtDNA. Although these strains are phenotypically identical, they clearly differ by the kind of mtDNA lesion that accounts for their respiratory-deficient state, and by activities taking place within the mitochondria. For example, the mit$^-$ strain is capable of mitochondrial protein synthesis, whereas the petite strains are not.

Our initial prejudice about the outcome of these experiments was that, if differential regulation exists among these cell types, it would be seen to varying degrees as a down-regulation of nuclear gene expression, either in response to respiratory deficiency in general, or to the loss of a genetically functional mitochondrial genome in particular. Arguably, such a response would be reasonable considering that the respiratory-deficient cells, and particularly the petites, should no longer require many of the nuclear-encoded mitochondrial proteins that function in oxidative phosphorylation and mitochondrial gene expression. Contrary to this expectation, however, we observed that, although there are indeed nuclear-derived transcripts whose abundance varies reproducibly in the four cell types examined, their abundance is generally *increased* in the respiratory-deficient cells, where in some cases they are most abundant in one or both of the petites. We have now extended these initial observations to include a wide variety of respiratory-deficient cells with different nuclear backgrounds.

Unusual, regulated transcripts from the nuclear ribosomal DNA repeat

The differential cDNA libraries we have constructed contain at least twenty and probably a much larger number of non-overlapping cDNAs that are derived from differentially expressed nuclear DNA transcripts. Of these, three have been unambiguously assigned to known sequences in the yeast nucleus: the 2μ plasmid, the cytochrome oxidase subunit VI gene, and the ribosomal DNA (rDNA) repeat. Although the cytochrome oxidase subunit VI gene is the only one of these sequences related to mitochondria in any obvious way, the present discussion will focus on the transcripts derived from the nuclear rDNA repeat, because these RNAs have unexpected properties given their rDNA origin, and their abundance suggests a possible control of nuclear rRNA synthesis that has led to the notion of cellular monitoring of mitochondrial apportionment, described below.

Figure 1 shows the relative abundance of a class of polydisperse RNAs in the ρ^+ and respiratory-deficient strains, measured by means of a cDNA probe (ρ19) derived from the

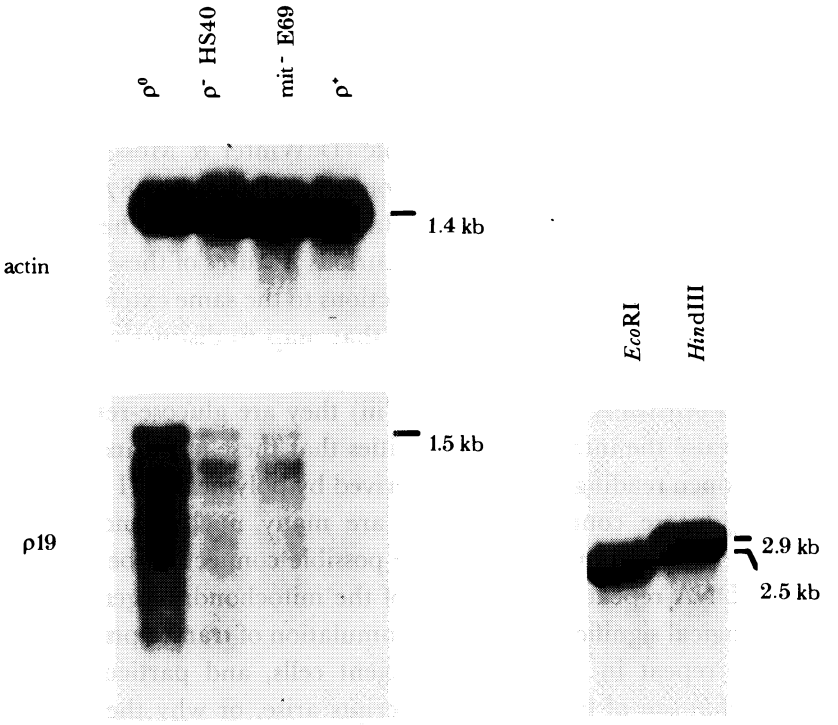

FIGURE 1. Transcripts derived from the nuclear rDNA repeat. A 200 bp cDNA probe, ρ19, was hybridized to a blot of poly(A)$^+$ RNA from the four strains indicated (bottom left); RNA loads were normalized to the levels of actin message (top left), which is the same in all the strains. A genomic Southern blot with the same cDNA probe is shown on the right. The ρ19 cDNA was sequenced and shown to have perfect homology to the spacer DNA region of the nuclear rDNA repeat located between the 5*S* gene and the start of transcription of the 37*S* precursor (see figure 2 and Warner (1981)). (These data are reprinted with permission, from Parikh *et al.* (1987).)

subtractive hybridization between mit$^-$ E69 and ρ^0 cells. Depending upon the preparation, the RNAs detected with this probe range in size from about 1500 nucleotides (NT) to less than 500 NT. These transcripts are barely detected in ρ$^+$ cells, and they are most abundant in the ρ^0 petite. From sequence analysis and preliminary transcript-mapping experiments, we have determined that these RNAs are derived from the so-called non-transcribed spacer (NTS) region of the rDNA repeat (figure 2); they are transcribed from the same strand as the 37*S* rRNA precursor, and extend at their 5′ end into the 5*S* region, and probably beyond (that is, 3′ to

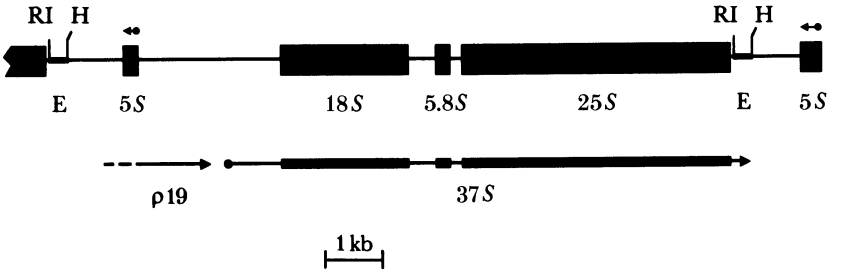

FIGURE 2. Location of ρ19 transcripts within the nuclear rDNA repeat: the organization of the nuclear rRNA genes. The direction of transcription of the 37*S* precursor, the 5*S* gene, and the ρ19 transcripts are indicated by the arrows, with the dot indicating the transcriptional initiation site. The 5′ and 3′ ends of the ρ19 transcripts have not been mapped precisely. RI and H are *Eco*RI and *Hin*dIII sites, respectively, that flank the polymerase I enhancer (E) (Elion & Warner 1986).

the 5*S* gene). We have detected an increased abundance of these NTS transcripts in every ρ^0 strain examined thus far, regardless of its nuclear background.

Although, by definition, NTS sequences were generally considered not to be transcribed, it is now apparent that the NTS regions of the nuclear rDNA repeat are indeed transcribed, but transcripts spanning these regions are very unstable (DeWinter & Moss 1986; Grummt *et al.* 1986; Henderson & Sollner-Webb 1986; Harrington & Chickaraishi 1987). To our knowledge, transcripts from the NTS regions do not accumulate in cells to anywhere near the level of abundance as we have found in ρ^0 petites. Other curious features of these yeast NTS transcripts are that (i) they are enriched in poly(A)$^+$ RNA fractions to the same extent as any typical yeast poly(A)$^+$ RNA; (ii) they have defined 3′ termini that map to distinctly different sites in the rDNA repeat than do the termination/processing sites associated with polymerase I rDNA transcripts (Kempers-Veenstra *et al.* 1986); and (iii) they are glucose-repressible.

These observations raise the intriguing possibilities that these NTS transcripts, which do not contain any extended open reading frames, are derived by polymerase II transcription and are subject to the same glucose control signals as are many nuclear-encoded mitochondrial proteins. This latter observation underscores the possible connection between the expression of NTS region of the rDNA repeat and the state of the mitochondrial genome.

What is the physiological significance of the accumulation of transcripts from the NTS regions of the nuclear rDNA repeat in respiratory-deficient cells, and particularly, in ρ^0 petites? Putting aside the mechanism of how these transcripts arise, or why they are so stable in ρ^0 petites, a simple notion for their increased abundance is that they reflect some underlying change in expression of the rRNA genes, which is related in some way to defects in mtDNA or its absence in ρ^0 petites.

The control of nuclear rRNA synthesis has been studied in considerable detail in many organisms, including yeast (Swanson *et al.* 1985; Elion & Warner 1986; Kempers-Veenstra *et al.* 1986). Estimates of the quantity of rRNA in yeast cells (*ca.* 85% of the total cellular RNA) shows a very precise relation, documented for a wide variety of growth conditions, to the doubling time: the longer the doubling time, the less rRNA per cell (Waldron & Lacroute 1975; Russokief & Warner 1981). Therefore, because petites grow slower than the ρ^+ on either glucose or non-repressing carbon sources, one would expect that rRNA synthesis should be correspondingly reduced in accordance with this general relationship. In this situation, the appearance of the unusual NTS transcripts might then be causally related to this control; for example, one could imagine that rRNA synthesis might somehow be attenuated because of enhanced transcription or stabilization of NTS sequences. This would represent a control mechanism for the regulation of rRNA synthesis that is unique, and one that would be superimposed on the well-documented controls engaged in the down-regulation of ribosome synthesis under growth-limiting conditions (Waldron & Lacroute 1975). On the other hand, the remarkable increase in the abundance of the NTS transcripts in the ρ^0 cell might reflect just the opposite, i.e. an increase in rRNA synthesis as part of an overall attempt by ρ^0 cells, albeit futile, to compensate for their respiratory-defective state. Experiments are now being done to decide between these possibilities.

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A model for mitochondrial copy control

Although we are far from understanding the full significance of the increased abundance in some respiratory-deficient cells of various transcripts of the nuclear genome, and especially those of the NTS regions of the rDNA repeat, we interpret this phenomenon to mean that these cells are attempting to compensate for the loss of a functional mitochondrial genome or the absence of mtDNA. What would such a compensation mean for wild-type cells? (Although petites can arise spontaneously with extraordinary frequency in *Saccharomyces cerevisiae* they are, nevertheless, mutants and are not part of the 'normal' life cycle or development of the yeast cell.) To begin to understand under what conditions a yeast cell might respond to the functional state or quantity of mtDNA, it is important to recognize that yeast cells seem to maintain about the same mass of mtDNA per cell regardless of the functional state of that DNA (Fukuhara 1969). Thus a petite whose mitochondrial genome may contain, for example, only a fraction of the sequences of the ρ^+ mitochondrial genome, amplifies those sequences to achieve about the same amount of mtDNA present in the ρ^+ cell (Nagley & Linnane 1972). Because mtDNA synthesis is not coupled to nuclear DNA replication (Williamson & Moustacchi 1972; Sena *et al.* 1975), the content of mtDNA could be determined, in a trivial sense, by its average replication rate and the cellular division time. A more biologically satisfying view, however, is that cells are able to measure their content of mtDNA in some direct way, regardless of sequence, and adjust that amount accordingly. Although such a process of genome maintenance does not have the elegance, say, of the mitotic apparatus in chromosome partitioning, it does offer a way in which budding yeast cells could guard against major inequalities in the allocation of mitochondria and mtDNA from one cell to another.

There is at least one stage in the yeast life cycle – the budding of diploid cells from zygotes – where there may be transient but dramatic change in the amount of mtDNA per cell, and perhaps in total mitochondrial mass as well. This is apparent from the observation that newly formed diploid cells become genotypically pure for mitochondrial alleles at a remarkably rapid rate, much faster than would be predicted for the random segregation of a large number of mtDNA molecules from zygotes to diploids. Without making any special assumptions about selective amplification or non-random segregation of mitochondrial genomes, a number of studies, which include pedigree analysis (Dujon *et al.* 1974; Dujon & Slonimski 1976; Strausberg & Perlman 1978) and direct measurements of mtDNA content in buds issued from diploids (Sena *et al.* 1976), suggest that an emerging diploid bud may contain only a small fraction of the total mtDNA in the mother zygote. Although these issues have been the subject of considerable discussion (see, for example, Birky & Skavaril 1976; Williamson *et al.* 1977; Dujon 1981), no clear picture of the physical unit of segregation of mtDNA has emerged. Despite this uncertainty, however, the segregation data are easily, but not exclusively, explained by the segregation of a small number of those units, and a proportional amount of mitochondrial mass, from the zygote to the emerging diploid bud. Additional support for this view comes from a recent study in which restriction-fragment length polymorphisms were used to measure the fraction of parental and recombinant forms of different mitochondrial loci in crosses. These experiments show a strikingly high fluctuation of variance and rapid purification of parental and recombinant forms of these loci in clones derived from individually dissected zygotes and first diploid buds (Zinn *et al.* 1988).

From the above considerations it follows that, if emerging diploid buds receive a relatively

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small fraction of mitochondria and mtDNA from the mother zygote, there must then be some amplification of these entities during bud maturation. Even with a broad distribution among individual cells in the amount of apportioned mitochondria and mtDNA, the cell would probably, in any case, have developed some mechanism to monitor apportionment and to make adjustments accordingly. Such adjustments would take the form of an increase in mitochondrial biogenesis and hence an increase in expression of certain nuclear genes. These increases could be significant in extent and the number of sequences involved, considering that perhaps 20% or so of the nuclear genome in yeast is devoted to mitochondrial biogenesis. The existence of a path of communication between the mitochondria and the nucleus that would allow the cell to monitor its contents of mitochondria and mtDNA implies that signals must shuttle between the organelles. Such signals could take the form of factors that partition between the genomes.

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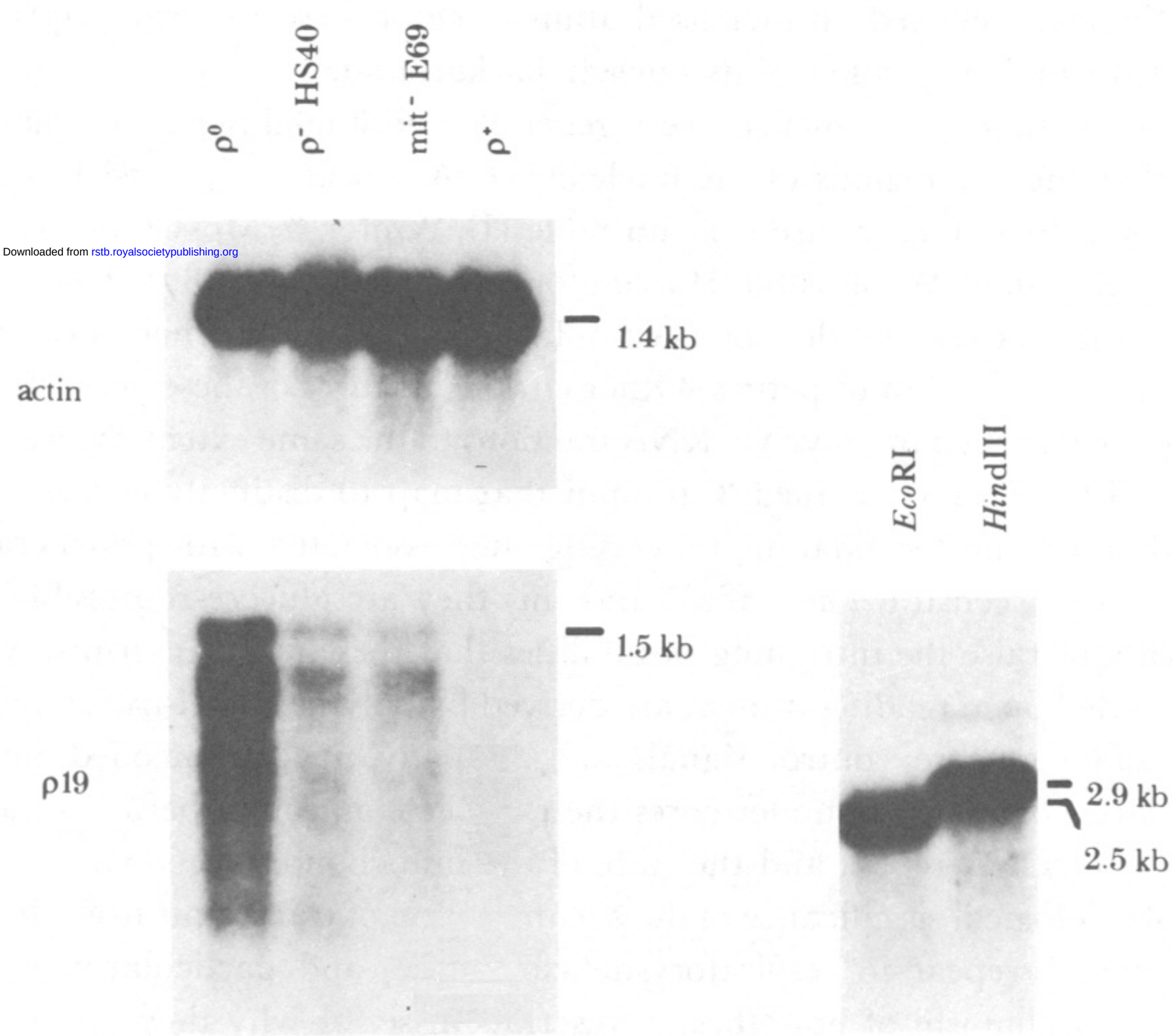

FIGURE 1. Transcripts derived from the nuclear rDNA repeat. A 200 bp cDNA probe, ρ19, was hybridized to a blot of poly(A)$^+$ RNA from the four strains indicated (bottom left); RNA loads were normalized to the levels of actin message (top left), which is the same in all the strains. A genomic Southern blot with the same cDNA probe is shown on the right. The ρ19 cDNA was sequenced and shown to have perfect homology to the spacer DNA region of the nuclear rDNA repeat located between the 5*S* gene and the start of transcription of the 37*S* precursor (see figure 2 and Warner (1981)). (These data are reprinted with permission, from Parikh *et al.* (1987).)