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Short Communication

Nuclear Association in Yeast of a Hybrid Vector Containing Mitochondrial DNA

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Summary. The hybrid vector pCP2, consisting of the bacterial plasmid pBR325, the nuclear gene Leu-2 of *Saccharomyces cerevisiae* and a fragment of mitochondrial DNA from *Cephalosporium acremonium*, was found to associate with the nucleus in a transformed strain of *Saccharomyces cerevisiae*. This was inducted by (1) efficient expression of the Leu-2 gene as evidenced by a short generation time on selective medium; (2) independence of Leu-2 gene expression from mitochondrial protein synthesis, since pCP2 was shown to replicate and to be expressed in petite mutants; (3) association of pCP2 with isolated DNA from nuclei as proved by transformation experiments with *E. coli*.

Key words: *Saccharomyces cerevisiae* – *Cephalosporium acremonium* – Mitochondrial hybrid vector – Nuclear association

As previously reported mitochondrial (mt) DNA may be used to develop cloning systems in eukaryotes (Stahl et al. 1982). During the course of such investigations a hybrid vector (pCP2) was constructed which consisted of the *Escherichia coli* plasmid pBR325, the *Saccharomyces cerevisiae* nuclear gene Leu-2 and the PstI-2 fragment from mtDNA of *Cephalosporium acremonium*. This vector proved to be a shuttle vector, replicating in *E. coli* as well as in yeast. Replication in the latter instance depended on the presence of the mtDNA fragment, which evidently carries a eukaryotic autonomous replicating sequence (ars) (Tudzynski and Esser 1982).

For practical exploitation of this vector it is necessary to know the site of replication and expression in the eukaryotic host. Specifically, does this shuttle vector associate either with the mitochondria or with the nucleus? Evidence that decides this alternative is based on the following experiments and considerations:

1. Efficient expression of the nuclear gene Leu-2 was evidenced by a short generation time (3.5 h) of transformed yeast cells on minimal medium (MM). This result corresponds to values obtained previously with nuclear associated plasmids (e.g. Stinchcomb et al. 1979). It seems rather improbable that pCP2 replicates and is expressed within the mitochondrion, since a lower rate of expression and therewith a longer generation time would be expected, due either to the difference of the genetic code between nuclear DNA and mtDNA or to the necessity to transport the Leu-2 gene product across mitochondrial membranes.

2. Independence of Leu-2 gene expression from mitochondrial protein synthesis was tested by analysing ethidium bromide induced petite mutants derived from *Saccharomyces cerevisiae* (AH22) containing pCP2. Among 48 petites 25 were leucine prototrophs (52%).

Since in the same experiment among 48 grandes colonies only 16 (37%) were prototrophic, the induction of the mitochondrial petites is not correlated with a loss of leucine prototrophy. In order to test if this phenotype (Leu^+) is due to free plasmids not integrated in nuclear DNA, bulk DNA from 5 different petides was used to transform *E. coli*. In all cases transformants were obtained in the range of 200–300/μg DNA. This demonstrates the existence of free independent plasmid DNA containing the prokaryotic part (pBR325 marker gene and replicon). The presence of these molecules within the petite cells was further shown by Southern hybridization of the petite bulk DNA with labelled pBR322 DNA (homologue to pBR325). Representative examples are given in Fig. 1.

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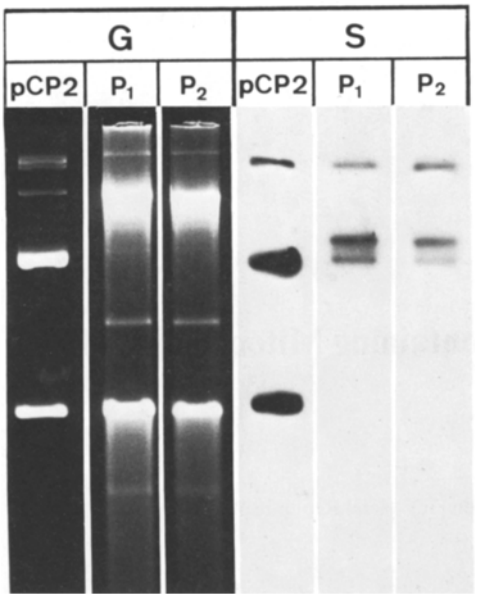

Fig. 1. *Saccharomyces cerevisiae:* gel electrophoreses (G) and corresponding Southern blots (S) of undigested bulk DNA of two petite mutants (P1, P2) derived from a pCP2 carrying strain. Reference: undigested pCP2 DNA; hybridization probe: [32 P]-dCTP-labelled pBR322 DNA; preparation of bulk DNA and hybridization according to Tudzynski and Esser (1982). The three lower bands of P1 and P2 which do not hybridize consist of 2 μm DNA (upper band) and killer RNA (middle and lower band). The middle band coincidently has the same electrophoretic mobility as the ccc form of pCP2. Despite this ccc DNA is not found in the P-mutants (caused by the mode of this specific "rapid" preparation) the identity of the hybridizing bands of P1 and P2 with pCP2 was proved by restriction analysis of *E. coli* transformants

3. Association of vector DNA (pCP2) with isolated nuclei was demonstrated in the following way: DNA from both, mitochondria and nuclei isolated from *S. cerevisiae* strain AH22, transformed with plasmid pCP2, was used to transform *Escherichia coli.*

As a reference, the same experiments were performed with the same yeast strain carrying a plasmid (pJDB248) containing no mitochondrial DNA but 2 μm DNA as replicon known to be associated with the nucleus (Beggs 1978). From the experiments summarized in Table 1 one may see, that in both cases nuclear preparations contain the vast majority of free plasmids. The fact that the mitochondrial preparations also contain free plasmids must be due to impurities, because the values obtained with the reference (the nuclear associated plasmid) were in the same range as those of pCP2.

In conclusion, the evidence presented here excludes an association of the mitochondrial hybrid vector pCP2 with the mitochondria and demonstrates its association with the nuclei. Recent results of Zakian and Kupfer (1982) confirm these findings: they proved that replication of a hybrid vector containing the *Xenopus* mitochondrial origin in yeast is controlled by the same genes which control chromosomal replication. From this it follows that, at least in yeast, a mitochondrial replicon is functional in the nucleus. Provided that the pCP2 vector behaves in the same way in *Cephalosporium*, by entering the nucleus instead of the mitochondrion, one may make use of this advantage in a homologous nuclear system for cloning nuclear genes.

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Table 1. Localization of hybrid vector pCP2 in cell organells, derived from transformation experiments in *E. coli.* DNA extracts from mitochondria and nuclei isolated from yeast cells carrying the hybrid plasmid pCP2 were used for bacterial transformation. As reference served the same yeast strain containing the hybrid vector pJDB248 (Beggs 1978) known to be associated with the yeast nucleus. Transformation of *E. coli* (Stahl et al. 1980); isolation of mitochondria (Tudzynski and Esser 1979); isolation of nuclei (Tudzynski 1983). Contamination of organelle preparations by free plasmid molecules was neglectable, as was monitored by transformation rate of plasmid pCE2 (Tudzynski and Esser 1982) added to the preparations after cell lysis

DNA preparation	*E. coli* transformants/μg DNA				
	pCP2			pJDB248	
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2
Mitochondria	37.0	19.5	19.8	0.7	66.5
Nuclei	410.0	786.0	671.0	13.6	291.0
Ratio (nuclei/mitoch.)	11.1	40.3	33.9	19.4	4.4