RECOMBINATION OF MITOCHONDRIAL DRUG-RESISTANCE FACTORS IN SACCHAROMYCES CEREVISIAE

D.Y. Thomas and D. Wilkie

Department of Botany, University College London.

Received January 17, 1968

Re-assortment of mitochondrially-located drug-resistance factors in the vegetative progeny of individual zygotes will be described and provides evidence of a recombinational process involving the genetic material of the organelle.

The specific inhibition of respiratory enzyme synthesis in yeast by certain protein inhibitors of bacteria was first reported by Linnane and his collaborators (see Clark-Walker and Linnane, 1967) and the isolation and characterization of spontaneous resistant mutants to some of these antibiotics is described in Wilkie et al (1967). A detailed account of a series of mutants resistant to erythromycin is given in Roodyn and Wilkie (1967) and Thomas and Wilkie (1968) in which the procedure for locating genetic factors for resistance in the so-called mitochondrial rho factor (generally assumed to be mitochondrial DNA (MDNA)) is fully described. Briefly this involves isolating the cytoplasmic petite mutant, in which there is effective loss of MDNA (see Mounolou et al, 1966), from those strains exhibiting non-chromosomal inheritance of resistant factors and demonstrating concomitant loss of the resistance factor in subsequent crosses to sensitive strains. In this way a number of rho factor mutants resistant to various antibiotics have now been

obtained and the present report concerns those resistant to the macrolide antibiotics erythromycin and spiramycin and the aminoglycoside paromomycin respectively. It may be emphasized that these drugs affect the protein-synthesizing system of the mitochondrion only and cause inhibition of cell growth only in media containing non-fermentable substrate.

METHODS

Genetically marked yeast strains of this laboratory were used. General techniques for crossing, zygote manipulation and determination of drug tolerance levels are detailed in Thomas and Wilkie (1968).

Since mitochondria are not synthesized and are not seen in cells of S. cerevisiae grown under anaerobic conditions (Lukins et al, 1967), although all genetic information for re-synthesis continues to be transmitted, it was thought that crossing of strains under anaerobic conditions would facilitate any recombinational process involving MDNA. Thus crosses were set up between anaerobic cultures of differently auxotrophic haploid strains in liquid minimal medium (Wickerham's) containing 4% glucose under a nitrogen atmosphere in a Fildes cylinder. Electron microscope studies show no evidence of mitochondrial structures in cells grown under our anaerobic conditions (unpublished results of Drs. R. Marchant and D. Smith of this laboratory).

The zygotes produced were usually in comparatively high numbers and were plated on solid minimal medium containing 2% glucose incubated aerobically for 2 days at 30°C, and the resulting prototrophic diploid colonies to which each zygote gave rise were picked off and suspended in water. Each clone of cells was then tested for drug resistance by dropping out onto the appropriate

drug series of plates. Some of the multiple-resistant recombinant clones were confirmed as such by testing cells grown on one antibiotic for ability to grow on the other drug or drugs. Confirmatory tests were also made on multiple-drug plates. This ruled out the possibility of clones of this type being comprised of mixtures of mitochondrial types either at the cell level or at the population level.

RESULTS AND DISCUSSION

The drop-out method of analysis of clones derived from individual zygotes enables an estimate to be made of the frequency of resistant and sensitive cells provided the cell density is not greater than about 10^3 per drop. In nearly all cases all cells within a clone derived from a zygote formed under anaerobic conditions seemed to contain mitochondria of one type only, and the segregation of types of clones is given in Table 1. It must be emphasized that these segregations and recombinations have taken place in <u>diploid</u> cells testifying to the non-chromosomal nature of the resistance factors.

If it is assumed that recombination involves crossing-over between MDNA strands carrying their respective resistance factors, it would appear that under anaerobic conditions only one MDNA strand survives in the zygote. However, the significant excess of multiple-sensitive recombinant clones in crosses 1, 3 and 4 must be accounted for. If it is further assumed that MDNA is circular, it will be appreciated that a single cross-over between two circles would give a single large loop while two or an even number of cross-overs would allow separation of two small circles.

This is not unlikely in view of Avers (1967) claim to have isolated circular MDNA filaments from yeast of different lengths. Thus a cross-over (or odd number of cross-overs) at any point

Table 1

Analysis of clones from individual zygotes in various crosses

Strains and crosses		Genotypes*	Clone types and number	Total clones
1-617		$E^s\ P^r$	$E^s\ P^r$ 25 $E^s\ P^s$ 31	80
X	(1)		$E^r\ P^s$ 19 $E^r\ P^r$ 5	
6-82		$E^r\ P^s$		
1-617		$E^s\ S^s\ P^r$	$E^s\ S^s\ P^r$ 49 $E^s\ S^r\ P^s$ 3 $E^r\ S^r\ P^s$ 25 $E^s\ S^r\ P^r$ 4 $E^s\ S^s\ P^s$ 14 $E^r\ S^s\ P^s$ 1	119
X	(2)			
6-82		$E^r\ S^r\ P^s$	$E^r\ S^r\ P^r$ 23	
1-601		$E^s\ S^s\ P^{r1}$	$E^s\ S^s\ P^r$ 49 $E^r\ S^r\ P^r$ 10 $E^r\ S^r\ P^r$ 47 $E^s\ S^r\ P^s$ 1 $E^s\ S^s\ P^s$ 21	156
X	(3)			
6-82		$E^r\ S^r\ P^s$		
1-619		$E^s\ S^{r1}$	$E^s\ S^r$ 2 $E^s\ S^s$ 152 $E^r\ S^r$ 40 $E^r\ S^s$ 1	195
X	(4)			
6-82		$E^r\ S^r$		

* E^s, sensitivity to 10 μg/ml erythromycin
E^r, resistance to >3 mg/ml erythromycin
S^s, sensitivity to 50 μg/ml spiramycin
S^r, resistance to 2 mg/ml spiramycin
P^s, sensitivity to 50 μg/ml paromycin
P^r, resistance to 1 mg/ml paromycin

---

would result in "diploid" MDNA heterozygous for resistance factors which would be expected to be recessive if they make altered mitochondrial ribosomes (discussed in Roodyn and Wilkie, 1967), the probable sites of action of these antibiotics. A conjoined configuration could be relatively stable and give only occasional resistant segregants within a clone as has been seen in some cases.

On the other hand, recombination may result from mixtures of the different MDNA strands within individual mitochondria, but if this generally applied one would expect much less distinction between clones and less uniformity within a clone

regarding mitochondrial type. These points may be resolved by further tests particularly tetrad analysis.

Crosses carried out aerobically yielded clones of mixed cells showing various proportions of the two parental types and plating on multiple-drug plates revealed very few recombinant cells of the multiple-resistant type.

It is perhaps premature to consider mapping resistance factors but it could be inferred from the data that the "genes" E and S for macrolide resistance are closely linked while these and the P "gene" are comparatively far apart on the mitochondrial genome.

The main features of this communication were given in a paper by Wilkie and Thomas read at the meeting of the British Genetical Society on 10 November 1967.

REFERENCES

Avers, C.J., Proc.Nat.Acad.Sci., 58, 620 (1967).

Clark-Walker, G.D. and Linnane, A.W., Biochem.Biophys.Res.Commun., 25, 8 (1967).

Lukins, H.B., Tham, S.H., Wallace, P.G. and Linnane, A.W., Biochem.Biophys.Res.Commun., 23, 363 (1967).

Mounolou, J., Jakob, H. and Slonimski, P.P., Biochem.Biophys. Res.Commun., 24, 218 (1966).

Roodyn, D.B. and Wilkie, D., The Biogenesis of Mitochondria, Methuen, London (1967).

Thomas, D.Y. and Wilkie, D., Genet.Res.Camb., 11 (1968). In the press.

Wilkie, D., Saunders, G. and Linnane, A.W., Genet.Res.Camb., 10, 199 (1967).