Erythromycin Inhibition of Sporulation in *Saccharomyces cerevisiae*

The sporulation process in the yeast, *Saccharomyces cerevisiae*, depends upon heterozygosis of the sex genes α and a[1,2] and on several environmental conditions among which aerobiosis, absence of organic nitrogen and of high concentration of fermentable carbohydrates play a key role[3].

Another important factor conditioning sporulation is the integrity of the mitochondrial structure. Respiratory deficient strains, whose phenotype is due to cytoplasmic or nuclear mutations[4,5], fail in fact to sporulate.

No evidence has so far been produced as to the role of mitochondrial protein synthesis in sporulation, although this aspect of the mitochondrial activity seems to be of great significance for other cellular functions[6]. This aspect of the problem was investigated by taking advantage of the specificity of the antibiotic erythromycin which is known to inhibit in vivo and in vitro mitochondrial protein synthesis[7,8].

In this paper data are reported indicating that mitochondrial protein synthesis plays a relevant role in the sporulation process, as suggested by the fact that the antibiotic blocks the process in erythromycin-sensitive but not in erythromycin-resistant strains of *S. cerevisiae*.

Materials and methods. Yeast strains: *S. cerevisiae*, strain 5675 α/*amet* 1290/*met* 1290, *his*5–2/*his*$^+$, *ade* 2–1/*ad*$^+$, was kindly supplied by Dr. S. SORA, Institute of Genetics, University of Milan (Italy).

Culture media: 23DHA (sporulation medium) and M (complete medium)[9].

Isolation and analysis of erythromycin resistant mutants: the mutants were isolated by plating 5×10^7 cells, on M solid medium containing 2mg/ml of erythromycin ethyl-succinate and 2% glycerol as carbon source. The mutants show an increase of the minimal inhibitory concentration from 1.5 mg/ml to 10 mg/ml. The mutation leading to ER^R phenotype segregates in a non-Mendelian fashion from the diploid ER$^R \times$ ER^S[10].

Growth and sporulation: growth and sporulation were carried out as previously described[9]. The count of asci was made after 5 days at 28 °C in an alternating shaker in the 23DHA medium. In each experiment, the fraction of cells which accomplished sporulation is determined. In standard conditions strain 5675 shows a sporulation frequency of 85%. For each treatment, the sporulation frequency is expressed as percentage of the control.

Respiration measurements: the oxidation of glucose and the endogenous respiration were measured as previously described[11].

Mitochondrial protein synthesis: the effects of erythromycin on the mitochondrial protein synthesis in wild type and erythromycin-resistant strain were studied according to LAMB et al.[8], by measuring the incorporation of L^{14}-C-leucine in isolated mitochondria.

Results and discussion. As shown in the Figure, sporification decreases with the increasing concentration of erythromycin in the sporulation medium: at 350 μg/ml of erythromycin, sporulation is reduced to the 50% of the control and concentrations above 4 mg/ml completely inhibit the process. These results cannot demonstrate a direct relationship between mitochondrial protein synthesis and sporulation, since the antibiotic could affect some other mechanism relevant to the process.

Some of the prominent possibilities were examined further.

The first one was that erythromycin could cause cytoplasmic mutation to ϱ^- and that, therefore, the cells fail to sporulate, being respiratory deficient. This possibility was eliminated by determining the frequency of ϱ^- cells

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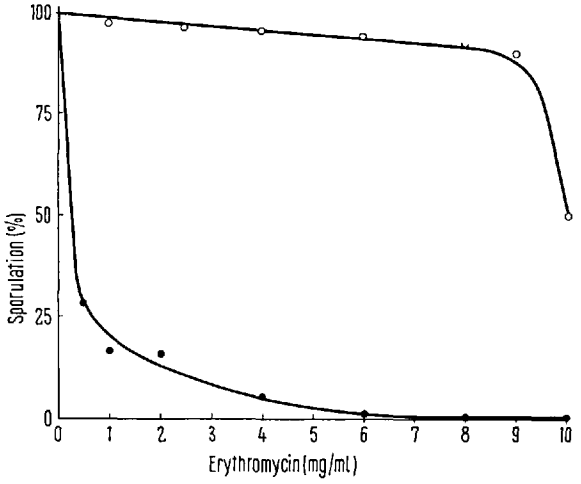
Table I. Effects of erythromycin on respiration of yeast cells during mitosis and sporulation

Cell	Erythromycin (mg/ml)	Respiration[b] Endogenous	Glucose (1%)
Mitotic	0	9.8	48
	1	9.6	51
	2	9.3	41
	4	9.4	48
	6	9.7	54
Meiotic[a]	0	9.4	48
	1	9.9	41
	2	10.3	48
	4	10.4	52
	5	9.8	47

[a] These results refer to cells assayed first 3 h after the transfer to 23DHA medium. Similar results were obtained at 6, 9, 12 and 16 h.
[b] Expressed as μl O_2/h/1 $\times 10^8$ cells.



Effects of erythromycin on sporulation in yeast: ○—○, 5675 ER^R; ●—●, 5675 ER^S.

induced by the highest concentration of erythromycin used in our experiments (10 mg/ml): such frequency (1.8%) turns out not to be different from the spontaneous (1.5).

We have successively analyzed the possibility that erythromycin inhibits cell respiration or ATP synthesis, giving rise to respiratory deficient phenocopies. In our experimental conditions, erythromycin interferes with the endogenous respiration or the oxidation of glucose only at high concentration (up to 10 mg/ml): in the range of the concentrations we used there is no effect on the respiration during the mitotic reproduction of the diploid or during its sporulation (Table I).

These observations cannot, however, eliminate the possibility that erythromycin blocks sporulation inhibiting other cellular functions critical for the process, and not the mitochondrial protein synthesis. We have thus studied the effects of the antibiotic on the sporulation of erythromycin-resistant strain (ER^R). As shown in the Figure, Er^R strain sporulates in presence of erythromycin even at concentrations of the antibiotic that inhibit completely the sporulation of the sensitive parent.

Since the lack of inhibition could depend upon an alteration of the cell permeability to the antibiotic, we have analyzed the effect of erythromycin on the mitochondrial protein synthesis in mitochondria isolated from the wild type and from ER^R strain. As shown in Table II, erythromycin inhibits protein synthesis of the mitochondria isolated from the sensitive parent but not from ER^R strain.

LINNANE et al.[10] have demonstrated that Er^R phenotype does not depend upon a change in the permeability of the mitochondrial membranes to the antibiotic. We have confirmed this observation in our strain by determining the effect of erythromycin on protein synthesis of mitochondria isolated from Er^R cells and partially disrupted by sonication[10]. In presence of 4 mg/ml of erythromycin, sonicated mitochondria incorporate 981 cpm/mg protein/min of L^{14}-C-leucine whereas the intact particles show an incorporation of 1241 cpm/mg protein/min of L^{14}-C-leucine. According to LINNANE et al.[10], we conclude therefore that Er^R phenotype rests on a mutational alteration of the sensitivity to the antibiotic of a component of the mitochondrial protein synthesizing machinery.

The data we have obtained could be summarized as follows: a) the only difference between the resistant and the sensitive strain appears to be the sensitivity of the mitochondrial protein synthesizing system to the antibiotic; b) erythromycin inhibits sporulation of erythromycin-sensitive parent but not of its erythromycin-resistant derivative. It could therefore be suggested that the inhibition of the sporulation by erythromycin in erythromycin-sensitive strain reflects the dependence of the process from the mitochondrial protein synthesis.

Whether this inhibition indicates that some of the proteins critical for sporulation are synthesized on the mitochondrial system, or that mitochondria mediate a coordinated switch on and off of the synthesis of proteins specific for sporulation, remains to be decided.

We favour at present the latter hypothesis, since it has been shown that resting cells of *S. cerevisiae* fail to be induced for several inducible enzymes in presence of erythromycin[6].

Table II. Effects of erythromycin on the incorporation of L^{14}-C-leucine by mitochondria isolated from erythromycin-sensitive (ER^S) and erythromycin resistant (ER^R) strain

Strain	Erythromycin (mg/ml)	^{14}C-leucine incorporation (counts/min/mg protein)
5432 ER^S	0	940
	1	50
	2	68
	4	50
	6	88
5432 ER^R	0	1241
	1	1041
	2	984
	4	1024
	6	1141
5432 Er^S-PD[a]	0	640
	2	59
	4	63
5432 Er^R-PD[a]	0	960
	2	880
	4	981

[a] PD, mitochondria partially disrupted by sonication according to LINNANE et al.[10].

Riassunto. Numerosi agenti chimici o condizioni fisiologiche ledono la funzionalità mitocondriale e contemporaneamente il processo di sporificazione nel lievito *Saccharomyces cerevisiae*. L'antibiotico eritromicina inibisce il processo di sporificazione nel lievito normale, ma é inattivo sulla sporificazione di mutanti Eritromicina resistenti. Ció suggerisce che la sintesi proteica mitocondriale svolge un ruolo rilevante nel processo di sporificazione.

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Distribution of Repetitious DNA in Human Chromosomes

One approach to the study of chromosome anatomy is the application of DNA/RNA or DNA/DNA hybridization principles to cytological preparations. This technique involves using the DNA molecules in cell nuclei or metaphase chromosomes as the immobilized 'receptors' and radioactive DNA or RNA as the mobile component for hybridization.

The 'satellite DNA' of the laboratory mouse which is easily separated by density gradient centrifugation is highly repetitious in base sequence[1]. This was one of the first DNA molecules exploited by PARDUE and GALL[2], and JONES[3] for in situ hybridization. These investigators found that the satellite DNA fraction is distributed in the heterochromatic regions near all centromeres with the exception of the *Y* chromosome which does not possess centromeric heterochromatin. It appears, therefore, that in other mammalian species some of the repetitious DNA also may be located in the heterochromatin regions. In most mammals, no specific satellite DNA fractions can be detected by cesium chloride density gradient analysis in an analytical ultracentrifuge[4,5]. However, all eukaryotes so far examined