INHIBITION OF *NEUROSPORA CRASSA* AND YEAST MITOCHONDRIAL PROTEIN SYNTHESIS BY RICIN, A TOXIC PROTEIN INACTIVE ON *E. COLI* PROTEIN SYNTHESIS

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1. Introduction

Ricin is a highly toxic protein isolated from *Ricinus communis* seeds. An inhibition of protein synthesis on the ribosomal level in ricin-treated rats was the first indication that this toxin acts on translation [1]. These results were confirmed by experiments on cells in culture [2] and on cell-free eukaryotic systems [3,4]. One of the two ricin polypeptide chains [5,6] or even ricin tryptic peptides [7] exhibit this inhibitory effect on in vitro systems. On the other hand, protein synthesis in *E. coli* cell-free system [8–10] is not reduced. These data indicate that only the eukaryotic type of ribosomes are susceptible to the toxins. Concerning the action of ricin on mitochondria, our preliminary results showed that ricin inhibits protein synthesis in yeast mitochondria [10] whereas Greco et al. [9] found no inhibition of rat liver mitochondrial protein biosynthesis by ricin. These results lead us to reinvestigate this problem. In this paper we show that intact ricin inhibits *Neurospora crassa* and yeast mitochondrial protein biosynthesis whereas it has no such action on an *E. coli* cell-free system.

2. Materials and methods

Ricin was prepared as previously described [11]. It was homogeneous in polyacrylamide disc gel electrophoresis and had no hemagglutinating effect [11].

Neurospora crassa mitochondria were isolated as previously described [12] and washed once with 1 mM EDTA, 10 mM Tris-acetate buffer, pH 7.5, 0.44 M sucrose.

A submitochondrial polypeptide-synthesizing system (S-30 fraction) was prepared and incubated with [^{3}H]phenylalanine at 37°C in the absence of exogenous messenger RNA as described [12]. Ricin was added one min after the start of the reaction. Aliquots of 20 μl were mixed with 1 ml of 5% trichloroacetic acid, heated at 95°C for 20 min, and passed through a 0.4 μm Millipore filter. The precipitate was washed with 5% trichloroacetic acid and the radioactivity counted in a Packard liquid scintillation counter.

Baker's yeast 'Yeast foam YF' strain, was grown, harvested and washed according to method A of Kellems and Butow [13]. Mitochondria were isolated sterilely and the purified mitochondria pellet was washed with 40 mM Tris-HCl buffer, pH 7.4, 250 mM sorbitol, 10 mM KCl. After homogenization in a Potter blendor, the concentration of mitochondrial proteins obtained was 50 mg per ml. The translation mixture was that of Lamb et al. [14]. After incubation 10 min at 37°C, 0.25 μCi/tube of each of the following [^{14}C]-labelled amino acids were added: L-valine, L-tyrosine, L-arginine and L-leucine. Ricin (20 μg/tube) was added to the reaction mixture 1 min after the start of protein synthesis. After 30 min at 32°C, an aliquot of 300 μl was withdrawn, and filtered through a serum albumin-coated 0.4 μm Millipore filter. The filter was washed once with a buffer containing all cold amino acids, then twice with 2 ml of ethanol and the precipitated radio-

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activity was measured by liquid scintillation counting.

The cell-free system from *E. coli* K12 was prepared according to the procedure of Nirenberg [15], using [^{3}H]phenylalanine. To this reaction either 20 μl of crude extract (preincubated 30 min at 37°C or not as indicated) or 20 μl of 105 000 *g* ribosomes and 10 μl of 105 000 *g* dialysed supernatant were added. In some experiments poly(U) (5 μl of a 1 mg/ml solution) was added. After 30 min at 37°C, 100 μl aliquots were removed and the radioactivity insoluble in hot trichloroacetic acid was counted in a Packard liquid scintillation counter.

3. Results

When ricin was added to the protein synthesizing system of *Neurospora crassa* mitochondria, incorporation of [^{3}H]phenylalanine into protein was strongly inhibited (fig.1). To verify the absence of cytoplasmic 77 S ribosomes in the mitochondrial preparation, cycloheximide (0.1 mg/ml) was added to the reaction mixture. Fig.1 shows that this contamination was negligible. To confirm this result phenol-extracted mitochondrial RNA was submitted to agarose gel electrophoresis. No contamination by cytoplasmic rRNA was found.

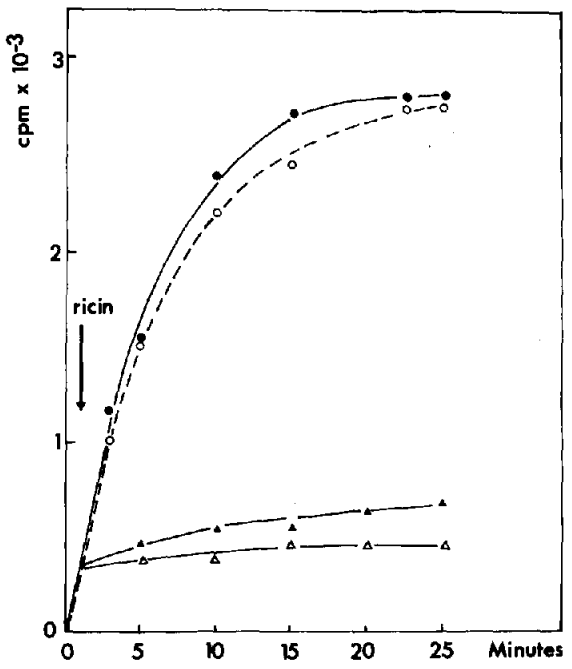

Fig.1. Effect of ricin on polypeptide synthesis by a *Neurospora crassa* submitochondrial system. (●——●) Control (○- - -○) Control plus cycloheximide (100 μg/ml) (▲——▲) Plus ricin (12 μg/ml) (△——△) Plus ricin (24 μg/ml).

Table 1 shows results obtained with yeast mitochondria. This system is not as clean as the *Neurospora crassa* one because it is inhibited to about 37% by cycloheximide (0.3 mg/ml) indicating contamination by cytoplasmic ribosomes. The amino acid incorporation is lower than with the *Neurospora crassa* S30 system but comparable to that obtained by other authors [16]. Nevertheless, with 20 μg ricin/ml a 100% inhibition was obtained.

Table 2 shows the results obtained using an *E. coli in vitro* system. No inhibition was detected even when very high doses of ricin (100 μg/ml) were added to a preincubated mRNA-free system (in presence of poly (U)) or to a non-preincubated system (in presence or in absence of poly (U)).

These results were confirmed by Springer and Grunberg-Manago (results not shown) who observed no inhibition of poly-valine synthesis when 166 μg/ml ricin was present in the poly (UG)-dependent *E. coli* in vitro system.

4. Discussion

So far the main functional criteria discriminating between prokaryotic and eukaryotic-cytoplasmic translational systems (e.g mechanism of peptide chain

Table 1
Radioactivity incorporated into yeast mitochondrial proteins in the presence of antibiotics and ricin

Addition to the mitochondria	cpm/mg[a]	% of total radioactivity
None	600	100
Cycloheximide	380	63
Cycloheximide plus chloramphenicol	10	1.7
Ricin	5	0.8

The incubation conditions are described in Materials and methods. The results shown are the average of 5 experiments.

[a]Counts per minute per mg of mitochondrial proteins after 30 min at 32°C.

Table 2
Ricin action on *E. coli* cell-free protein synthesis

	Reaction mixture	[^{3}H]phenylalanine incorporated (dpm)
Preincubated mRNA-free system	Complete	3218
	No poly (U)	153
	Complete, plus ricin	3186
	Plus ricin, no poly (U)	161
Non-preincubated system	Complete	4264
	No poly (U)	1882
	Complete, plus ricin	4188
	Plus ricin, no poly (U)	1776

initiation, specificity of ribosome interaction with elongation factor G, sensitivity to antibiotics) have placed the mitochondrial protein synthesizing system unequivocally in the prokaryotic class (see [17]). Here we report an exception, to this rule: to our knowledge the toxic protein ricin is the first known inhibitor of both cytoplasmic and mitochondrial protein synthesis at least in fungi, which is inactive in a prokaryotic (*E. coli*) cell-free system. The situation might be different in the rat liver system, where an inhibition of mitochondrial protein synthesis by ricin was not observed [9].

Ricin has been shown to interfere with the elongation of already initiated polypeptide chains [18,19]. An 8 S complex, consisting of 5 S RNA and L3 protein, obtained by treatment of 60 S mammalian cytoplasmic ribosomes with EDTA [20–22] has some GTPase activity [21]. Since one of the two polypeptide chains of ricin (A-chain) [4], inhibits this activity [23] the 8 S complex could possibly be the target of ricin action.

Whatever the exact mechanism of ricin action may be, it is evident that the otherwise bacteria-like mitochondrial ribosome in fungi is more related to the eukaryotic-cytoplasmic ribosome in its ricin recognition site.

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