EFFECT OF ANTIBACTERIAL INHIBITORS ON PROTEIN SYNTHESIS IN ISOLATED FLIGHT MUSCLE MITOCHONDRIA OF THE BLOWFLY *LUCILIA CUPRINA*: PHYLOGENETIC IMPLICATIONS

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1. Introduction

While mitochondria from all organisms have a similar basic structure and enzymic pattern, it is becoming clear that there are considerable differences in the genetic apparatus of mitochondria of organisms of different phylogenetic levels. If fungal and animal mitochondria are compared, there is a similarity within each group with respect to their ribosomes [1] and DNA content [2], but there are large differences between these groups when these characteristics are compared. Protein synthesis in all mitochondria is resistant to the cytoplasmic protein synthesis inhibitor cycloheximide, and sensitive to the anti-bacterial inhibitor D(−)*threo* chloramphenicol. The sensitivity to various other anti-bacterial inhibitors of protein synthesis by isolated yeast and rat liver mitochondria has been characterised. Yeast mitochondria are sensitive to several inhibitors affecting both the small (e.g., neomycin, paromomycin) and large (e.g., erythromycin, lincomycin) ribosomal subunits, while rat liver mitochondria are resistant to these inhibitors [3–5]. In a previous report we showed that mitochondria from the blowfly *Lucilia cuprina* are rat-like in being resistant to erythromycin [6]. We show here that protein synthesis of mitochondria from *L. cuprina* is sensitive to some small ribosomal subunit inhibitors (yeast-like) and resistant to some large ribosomal subunit inhibitors (rat-like).

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2. Materials and methods

The blowfly *Lucilia cuprina* was reared aseptically [7]. Flight muscle mitochondria were isolated from newly emerged axenic adult blowflies in 0.154 M KCl + 1 mM EDTA (pH 7.4) as described previously [6]. Sterile procedures were used and in no experiment reported was the level of contamination more than 50 bacteria/incubation. Freshly prepared mitochondria (approx. 1 mg protein) were preincubated at 30°C for 5 min in 1 ml of a medium (pH 7.4) containing 65 mM KCl, 10 mM potassium phosphate, 1 mM EDTA, 18 mM $MgSO_4$, 10 mM HEPES buffer, a mixture of 19 amino acids (without leucine) each 0.05 mM, ATP generating system (4 mM ATP, 5 mM phosphoenolpyruvate, 20 μg pyruvate kinase) and inhibitor [8]. The 15 min incubation was initiated by adding 0.5 μCi [U-^{14}C]leucine (specific activity 331 mCi/mmole, Amersham). In some experiments mitochondria were given a hypotonic shock by resuspending them finally in water, and standing them on ice for 15 min, before adding them to the incubation mixture.

[^{14}C]Leucine incorporation into mitochondrial protein was determined as described previously using hot and cold trichloroacetic acid washes, and extraction in ethanol, ethanol/ether, and ether [6]. In all experiments zero time controls terminated with trichloroacetic acid gave background radioactivity.

Inhibitors were obtained from the following

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sources: D(−)*threo* chloramphenicol, cycloheximide, neomycin (mixture of B & C), (Sigma); lincomycin, neamine (Gift from Upjohn Co., Kalamazoo, Michigan, USA); paromomycin (Gift from Parke, Davis, and Co., Detroit, Michigan, USA); kanamycin sulphate (Gift from Bristol Labs. Pty. Ltd., Brookvale, NSW, Australia). All inhibitors were dissolved in sterile distilled water.

3. Results

Two series of experiments are summarised in tables 1 and 2. Table 1 shows the effect on incorporation of [^{14}C]leucine into intact mitochondria of the cytoplasmic synthesis inhibitor cycloheximide, and anti-bacterial inhibitors chloramphenicol, lincomycin, oleandomycin, paromomycin, neomycin, neamine, and kanamycin. Cycloheximide produced less than 10% inhibition of [^{14}C]leucine incorporation confirming that there is no significant contribution by a cytoplasmic component to the observed incorporation; cycloheximide is an effective inhibitor of cytoplasmic protein synthesis in *L. cuprina* [9]. D(−)*threo* chloramphenicol produced strong inhibition above 50 μg/ml. The inhibitors oleandomycin and lincomycin, which affect the large ribosomal subunit in bacteria, produced less than 10% and slight inhibition respectively. Inhibitors which affect the small ribosomal subunit of bacteria fell into two classes. Paromomycin and neomycin produced strong inhibition, while neamine and kanamycin were less effective inhibitors.

Table 2 shows the effect of some of the above inhibitors on the incorporation of [^{14}C]leucine into mitochondria given a hypotonic shock. The effect on inhibition by cycloheximide, chloramphenicol, oleandomycin, and lincomycin was similar to that observed with intact mitochondria, while both paromomycin and neomycin were slightly more inhibitory.

4. Discussion

The results reported here indicate that blowfly mitochondria have a distinctive pattern of resistance and sensitivity to the anti-bacterial inhibitors tested. When the anti-bacterial inhibitors affecting the large ribosomal subunit are considered, *L. cuprina* shows a rat-like spectrum in being resistant to erythromycin

Table 1
The effect of various inhibitors on the incorporation in vitro of [^{14}C]leucine into protein of intact flight muscle mitochondria of *Lucilia curpina*

Inhibitor (μg/ml)	Incorporation as % of control mitochondria							
	Cytoplasmic inhibitor	Bacterial inhibitor						
		Large ribosomal subunit			Small ribosomal subunit			
	CYC	CAP	OLE	LINC	PAR	NEO	NEA	KAN
0	100	100	100	100	100	100	100	100
10	100	91	–	–	82	–	–	–
20	–	75	96	–	68	46	96	96
50	107	57	96	92	57	35	88	92
100	94	46	95	80	48	30	69	96
150	110	38	–	–	–	–	–	–
200	97	30	93	74	–	30	75	89
300	–	–	91	67	–	–	67	82
400	–	19	–	–	–	–	–	–
500	94	–	–	–	–	–	–	–

CYC: cycloheximide; CAP: D(−)*threo* chloramphenicol; OLE: oleandomycin; LINC: lincomycin; PAR: paromomycin; NEO: neomycin; NEA: neamine; KAN: kanamycin. Incubation conditions are described in the Methods. Control incorporation in these experiments was 5020 cpm/mg protein/15 min (mean of four experiments).

Table 2
The effect of various inhibitors on the incorporation in vitro of [^{14}C]leucine into protein of flight muscle mitochondria resuspended in water

Inhibitor (μg/ml)	Incorporation as % of control mitochondria					
	Cytoplasmic inhibitor	Bacteriol inhibitor				
		Large ribosomal subunit			Small ribosomal subunit	
	CYC*	GAP	OLE	LINC	PAR	NEO
0	100	100	100	100	100	100
10	–	–	–	–	–	40
20	90	66	97	–	71	25
50	101	58	93	85	39	14
100	111	41	–	82	27	9
200	109	26	88	73	16	–
300	–	18	–	50	–	–
400	–	18	–	–	–	–
500	99	–	–	–	–	–

* Abbreviations are defined in table 1. Incubation conditions are described in Materials and methods. Control incorporation in these experiments was 4180 cpm/mg protein/15 min (mean of three experiments).

and slightly sensitive to lincomycin [3,6]. Yeast mitochondria are sensitive to erythromycin, oleandomycin, and lincomycin [10]. Erythromycin has been used commonly in various comparative studies, and higher organisms appear to be resistant to this inhibitor while lower organisms are sensitive [3]. Another insect, the colorado potato beetle, has mitochondria that are resistant to erythromycin [11]. The only insect whose mitochondrial ribosomes have been studied is the locust; this insect has a 'mini' mitochondrial ribosome [12]. It has been suggested that organisms with a 'mini' mitochondrial ribosome (55 S–60 S) are erythromycin resistant and those with a yeast-like mitochondrial ribosome (70 S–74 S) are sensitive [5]. The results obtained with insects are probably consistent with this, but there appear to be two possible exceptions to the generalisation; mitochondria of BHK-21 cells [13] and mitochondria of the trypanosomatid *Crithidia luciliae* [14] are both sensitive to erythromycin although both have 'mini' mitochondrial ribosomes.

There is only one report on the comparative effect of aminoglycoside antibiotics, which affect the small ribosomal subunit [4]. Rat liver mitochondria are resistant to all of the aminoglycoside antibiotics studied here, while yeast mitochondria are sensitive to neomycin and paromomycin, and slightly sensitive to neamine and kanamycin [4]. Results reported here for *L. cuprina* mitochondria are analogous to those found with yeast mitochondria.

The nature of the resistance in the rat mitochondria, at least for the large ribosomal subunit inhibitors, is still controversial. There is evidence which suggests two different mechanisms for resistance, one emphasising an alteration of the structure of the mitochondrial ribosome [5,15], and the second, changes in mitochondrial permeability [13,16]. Regardless of the nature of the resistance mechanism. These studies on blowfly mitochondria make clear that phylogenetic alterations in the resistance pattern for small and large ribosomal subunit inhibitors may occur independently of each other.

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