DIFFERENTIATION IN VITRO BY PHENANTHRENE ALKALOIDS OF YEAST MITOCHONDRIAL PROTEIN SYNTHESIS FROM RIBOSOMAL SYSTEMS OF BOTH YEAST AND BACTERIA.

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The antibacterial antibiotics chloramphenicol, lincomycin and the macrolides erythromycin, carbomycin, spiramycin and oleandomycin act by inhibiting bacterial protein synthesis. The same antibiotics also inhibit the mitochondrial protein synthesising system of Saccharomyces cerevisiae both in vivo and in vitro without affecting the cytoplasmic ribosomal system (Clark-Walker and Linnane, 1966; Lamb, Clark-Walker and Linnane, 1968). Conversely, cycloheximide which inhibits the synthesis of protein by the cytoplasmic ribosomes of eucaryotic cells, is without effect on bacterial protein synthesis (Siegel and Sisler, 1966; Young, Robinson and Sacktor, 1963), and also has no effect on the mitochondrial protein synthesising system of S. cerevisiae (Lamb et al., 1968). Based on these observations it has been suggested that the mitochondrial protein synthesising system is similar or identical to that occurring in bacteria. However, this communication reports that the yeast mitochondrial protein synthesising system differs not only from the yeast cytoplasmic ribosomal system but also from the Escherichia coli ribosomal system.

The three phenanthrene alkaloids, tylophorine, tylocrebrine and cryptopleurine inhibit growth and protein synthesis in tumour cells (Donaldson, Atkinson and Murray, 1968), and are herein shown to act directly on the ribosome and to differentiate between the protein synthesising systems of yeast mitochondria, of yeast cytoplasmic ribosomes and of ribosomes from E. coli.

METHODS

References to the isolation and characterisation of tylophorine, tylocrebrine and cryptopleurine are given by Donaldson et al. (1968). A haploid strain of S. cerevisiae, 21-10A, was grown aerobically at 28° on a 1% glucose, 1% Difco yeast extract, 0.5% peptone, salts medium, and cells were harvested in early stationary phase of growth. E. coli strain AB 264 was grown aerobically at 28° on a minimal salts medium supplemented with 1% glucose, 0.2% peptone, and cells were harvested in logarithmic growth phase. In the isolation of cytoplasmic ribosomes and pH 5 enzymes, from yeast or E. coli, cells were broken by grinding with powdered alumina (Nirenberg, 1964), and in the isolation of yeast mitochondria cells were broken by subjecting to osmotic shock protoplasts prepared by treating cells with snail-gut enzymes (Duell, Inoue and Utter, 1964, as modified by Lamb et al., 1968). The subsequent isolation of mitochondria, cytoplasmic ribosomes and pH 5 enzymes and the measurement of the incorporation of C^{14} leucine into protein were as described by Lamb et al. (1968).

RESULTS AND DISCUSSION

All three phenanthrene alkaloids inhibited the growth of S. cerevisiae; cryptopleurine, tylocrebrine and tylophorine caused 50% inhibition of growth at 2 μM, 6 μM and 100 μM respectively. Growth was completely inhibited by 10 μM cryptopleurine or 30 μM tylocrebrine, but only partial inhibition could be obtained with tylophorine due to its limited solubility (100 μM). This is the same sequence of inhibitory activity as that previously observed with Ehrlich ascites cells (Donaldson et al., 1968).

The effects of tylophorine, tylocrebrine and cryptopleurine on the incorporation of (^{14}C)-leucine into protein by yeast cytoplasmic ribosomes, yeast mitochondria and E. coli ribosomes are shown in Table 1. All three alkaloids were effective in inhibiting the yeast cytoplasmic ribosomal system, whereas only tylocrebrine and cryptopleurine inhibited the mitochondrial system; even so, much higher concentrations of these alkaloids were required to cause a degree of inhibition of the mitochondrial system comparable to that observed with the cytoplasmic

TABLE 1

EFFECTS OF CRYPTOPLEURINE, TYLOCREBRINE AND TYLOPHORINE ON THE INCORPORATION OF LEUCINE INTO PROTEIN BY YEAST CYTOPLASMIC RIBOSOMES, YEAST MITOCHONDRIA AND E. COLI RIBOSOMES.

		% Inhibition of Incorporation of (^{14}C)-Leucine into Protein		
Inhibitor	Conc. (μM)	Yeast Cytoplasmic Ribosomes	Yeast Mitochondria	E. coli Ribosomes
Cryptopleurine	0.2	25	0	0
	1	64	0	2
	2	59	20	6
	4	71	60	8
	20	70	81	19
	50	70	87	37
	100	70	96	55
Tylocrebrine	0.4	38	0	0
	2	56	6	+2
	10	71	42	+1
	40	84	64	8
	100	80	75	12
Tylophorine	2	12	0	+4
	10	38	0	+5
	50	67	2	0
	100	72	8	5

The incorporation of (^{14}C)-leucine into protein was measured as described by Lamb et al. (1968). Results are expressed as % inhibition of incorporation as compared with control incubations. Specific activities of the protein synthesising systems of mitochondria, yeast cytoplasmic ribosomes, and E. coli ribosomes were 20, 35 and 200 μμmoles (^{14}C)-leucine/mg protein/20 min. incubation respectively.

ribosomes. Very high concentrations of cryptopleurine were required to produce any inhibition of the E. coli ribosomal system, whereas both tylophorine and tylocrebrine had no signif-

TABLE 2

THE EFFECT OF CRYPTOPLEURINE AND TYLOCREBRINE ON THE CYTOPLASMIC RIBOSOMES OF E. COLI OR S. CEREVISIAE WHEN ACTIVATED BY EITHER HOMOLOGOUS OR HETEROLOGOUS pH 5 ENZYMES.

Enzyme Source	Alkaloid (μg/ml)		% Inhibition
Yeast ribosomes + Yeast pH 5	Cryptopleurine	(1)	90
	Tylocrebrine	(2)	71
Yeast ribosomes + E. coli pH 5	Cryptopleurine	(1)	83
	Tylocrebrine	(2)	82
E. coli ribosomes + E. coli pH 5	Cryptopleurine	(20)	12
E. coli ribosomes + Yeast pH 5	Cryptopleurine	(20)	17

Results are expressed as the percentage inhibition of (^{14}C)-leucine incorporation into protein. Specific activities of the protein synthesising systems of yeast ribosomes + yeast pH 5 enzymes, yeast ribosomes + E. coli pH 5 enzymes, E. coli ribosomes + E. coli pH 5 enzymes, E. coli ribosomes + yeast pH 5 enzymes were 100, 85, 500, 450 μμmoles (^{14}C)-leucine/mg protein/20 min. incubation respectively. In the absence of pH 5 enzymes the activities of the isolated ribosomes were 5-15% of those of the complete system.

icant effect. Thus, the three alkaloids distinguish the mitochondrial protein synthesising system from both the yeast cytoplasmic ribosomal system and the bacterial ribosomal system.

The preparations of cytoplasmic ribosomes from yeast and E. coli were dependent upon the pH 5 enzyme fraction for their ability to incorporate amino acids into protein. However, homologous pH 5 enzyme fractions were not specifically required for incorporation activity, and the pH 5 enzymes from either organism were freely interchangeable. Table 2 shows that cryptopleurine and tylocrebrine react directly with the ribosome; thus irrespective of the source of the pH 5 enzymes the two alkaloids strongly inhibited the yeast ribosomal system and were without effect on the E. coli system. Further support for this conclusion was provided by experiments in which yeast ribosomes

were pre-incubated with cryptopleurine (4μg/mg protein), and were then washed. These ribosomes were then shown to be no longer capable of functioning in the incorporation of amino acids into protein, although ribosomes pre-incubated in the absence of cryptopleurine retained their activity. Thus cryptopleurine irreversibly affects the ribosome, presumably by being bound to it.

Yeast mitochondrial, bacterial and chloroplast protein synthesising systems are inhibited by the antibacterial antibiotics chloramphenicol, lincomycin and the macrolides, while yeast cytoplasmic ribosomes are unaffected by these antibiotics (Linnane and Stewart, 1967). Since chloroplast and bacterial ribosomes are of the 70S type in contrast with the cytoplasmic ribosomes of eucaryotic cells which are of the 80S type, it was previously proposed that mitochondria are likely to contain 70S type ribosomes (Clark-Walker and Linnane, 1966). However, direct evidence on the size of the mitochondrial ribosome and on the size and number of its RNA components is controversial. There is some agreement that the high molecular weight RNA species of yeast mitochondria (Rogers et al., 1967; Wintersberger, 1967) and of Neurospora mitochondria (Küntzel et al., 1967; Rifkin et al., 1967) resembles that from E. coli ribosomes in sedimentation characteristics, but O'Brien and Kalf have reported that rat liver mitochondria contain only a 28S RNA species. Rifkin et al. (1967) have described the ribosome of Neurospora mitochondria as an 80S particle, comparable in size to the cytoplasmic ribosome, whilst Küntzel and Noll (1967) reported a smaller 70S type ribosome; O'Brien and Kalf (1967) report a third situation in which a 55S ribosome occurs in rat liver mitochondria.

Since the yeast mitochondrial protein synthesising system differs from both the E. coli 70S and the yeast cytoplasmic 80S system in its sensitivity to the phenanthrene alkaloids it may also differ from them in other properties, including size. However, even if ribosomes of different types are similar in size they may differ in other properties. The present work serves to emphasise the differences between systems which have been recently considered to be very similar if not identical. Indeed, recent experiments indicate that even mitochondria from different species have different protein synthesising systems (Firkin and Linnane,

1968a). Thus, rat and rabbit liver mitochondria both *in vivo* and *in vitro* differed from yeast mitochondria in their spectrum of response and sensitivity to the antibacterial antibiotics, and the amino acid incorporating system of only the latter was inhibited by lincomycin and erythromycin (Firkin and Linnane, 1968a,b).

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