Biochimica et Biophysica Acta, 1000 (1989) 365–366
Elsevier

Commentary by

Anthony W. Linnane

Centre for Molecular Biology and Medicine, Monash University, Clayton (Australia)

on 'Chloramphenicol inhibition of the formation of particulate mitochondrial enzymes of *Saccharomyes cerevisiae*'
by M. Huang, D.R. Biggs, G.D. Clark-Walker and A.W. Linnane
Biochim. Biophys. Acta. 114 (1966) 434–436

The 1966 short communication was concerned with our early faltering steps on the development of an experimental approach to the biogenesis of yeast mitochondria. At that time, petite respiratory deficient cells had been genetically characterised by Ephrussi and his colleagues in the late 1940's as cytoplasmically inherited mutants which lacked a functional mitochondrial respiratory chain. Ephrussi's work was subsequent to Avery's recognition of DNA as the hereditary substance of chromosomes but prior to the work of Watson and Crick and the double helix. About the time of our publication, the existence of mitochondrial DNA was recognised, but its significance was not understood, no gene products were known to be encoded in this DNA. Changes in the buoyant density of mtDNA in cytoplasmic petite mutants compared with wild-type cells was a current topic of research. However, it was even controversial as to whether there was a mitochondrial protein synthesising system. Studies of the biosynthesis of mitochondria were at the earliest stage of development.

Anthony W. Linnane

The results described in the paper showed that by growing yeast in the presence of chloramphenicol, the membrane-bound mitochondrial cytochromes a, a_3 and b, were no longer synthesised. Effectively a phenocopy of the cytoplasmic petite mutant was obtained by growing cells in the presence of chloramphenicol. The paper established that the cytoplasmic ribosomal system was not affected by chloramphenicol as the yeast cells grew normally on fermentable substrates, such as glucose, while being rendered respiratory deficient in the presence of the antibiotic. Chloramphenicol was shown not to induce petite mutants, as on transfer of the cells to chloramphenicol-free media the respiratory enzymes were re-synthesised. We interpreted this work in terms of the mitochondria possessing a specific protein synthesising system which was bacterial-like in characteristics, being sensitive to inhibitors of bacterial protein synthesis, while the cytoplasmic ribosomal system was different and unaffected by the anti-bacterial antibiotics. Cytochromes a, a_3 and b were proposed as products of the mitochondrial protein synthesising system. The action of chloramphenicol to that time was indeed controversial; proposals for its effects on eukaryote cells included considerations that it not only acted directly as an inhibitor of the *cytoplasmic* ribosomal system but also that it was a direct inhibitor of respiration.

Soon after this paper, we showed that a number of antibiotics which were inhibitors of bacterial protein synthesis, for example, the tetracyclines, erythromycin

and paromomycin, were also inhibitors of mitochondrial protein synthesis both in vivo and in vitro, as we also showed for chloroplast protein synthesis. Conversely, we reported cycloheximide to be a specific inhibitor of the cytoplasmic ribosomal system.

The demonstration of the in vivo effect of chloramphenicol also dealt a telling blow to the canard that the observed in vitro protein synthesis by mitochondria was an artefact due to bacterial contamination.

Chloramphenicol and cycloheximide were then established as the standard reagents for the differential inhibition of mitochondrial and cytoplasmic ribosomal protein syntheses. These reagents continue to be widely used in cell biology studies to differentiate the mitochondrial (and chloroplast) protein-synthesising system from the cytoplasmic ribosomal system and the relevant gene products of the systems.

Another far-reaching consequence of our observations was the opening up of the whole field of mitochondrial biogenesis. The work quickly led to our discovery of the first mitochondrially encoded mutants; these were phenotypically characterised by their resistance or sensitivity to antibacterial antibiotics. Conceptually, it readily followed that a number of other antibiotics known to affect the mitochondrial respiratory system such as antimycin A and oligomycin could similarly be used to isolate further mitochondrial mutants. Mitochondrially encoded mutants resistant to oligomycin and antimycin A were isolated by several laboratories; in vivo and in vitro effects of the mutations on mitochondrial ATP synthase and cytochrome *b* were later demonstrated. The availability of these mutants enabled a rational approach to be developed for the study of cytoplasmic genetics and the recognition of the nature of the gene products encoded in mitochondrial DNA.

The years 1965 to 1967 were indeed an exciting time in our laboratory and provided the basis for over 20 years of interest and work on mitochondrial biogenesis by our laboratory. The culmination of these studies was the elucidation of the first physical map of genetic loci in yeast mitochondrial DNA (the first for any organelle genome). The development of this work and its outcome have been summarised by Linnane and Nagley (1978) Plasmid 1, 324–345.

Correspondence: A.W. Linnane, Centre for Molecular Biology and Medicine, Monash University, Clayton, Victoria 3168, Australia.

BBA 91098

Chloramphenicol inhibition of the formation of particulate mitochondrial enzymes of *Saccharomyces cerevisiae*

Current work in this laboratory is concerned with the problem of mitochondrial biogenesis in yeast. In the course of these studies we have found that chloramphenicol inhibits amino acid incorporation by isolated yeast mitochondria. This finding confirms the results of others working with mitochondria isolated from a variety of sources[1–3]. However, chloramphenicol with similar concentrations to those used with mitochondria does not inhibit amino acid incorporation by isolated animal[4] and yeast[5,6] cytoplasmic ribosomes. We were therefore led to consider the possibility that chloramphenicol exerts its inhibitory action on higher organisms by a selective inhibition of mitochondrial protein synthesis *in vivo*.

The present communication reports the effect of chloramphenicol on growing cells of *Saccharomyces cerevisiae*. It is shown that this yeast forms only trace amounts of cytochrome *b* and no longer forms cytochromes aa_3 in the presence of the drug although the growth rate and the final yield of cells are unchanged provided the growth medium contains high concentrations of fermentable substrate (5 % glucose).

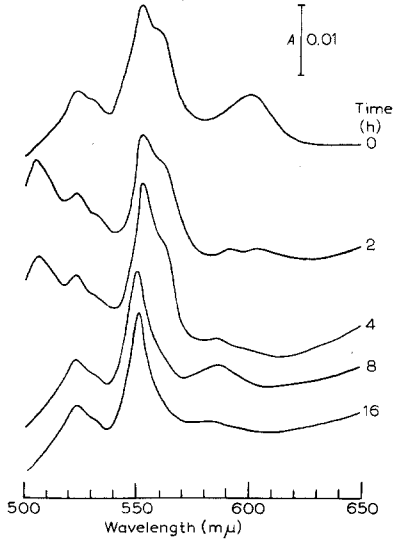

Fig. 1. *S. cerevisiae* cells were grown aerobically for 16 h in 5 % glucose medium and then transferred to medium containing 4 mg/ml chloramphenicol. Samples were taken at the time intervals shown, and the spectra recorded in a Cary Model 14 Spectrophotometer fitted with a Scattered Transmission Accessory (Model 1462). The cells were suspended in 30 % (v/v) glycerol to a final cell density of 15–20 mg dry wt. of cells per ml of suspension, and reduced with sodium dithionite. The samples were compared with a reference gel suspension consisting of flour (800 mg) and soluble starch (75 mg) suspended in 15 ml of water. The reference gel showed no absorption peaks over the range of wavelengths used for the measurements.

Biochim. Biophys. Acta, 114 (1966) 434–436

S. cerevisiae cells were grown aerobically at 30° in a medium[7] containing 5 % glucose and 4 mg chloramphenicol/ml medium. A progressive alteration occurred in the visible absorption spectrum of the yeast as illustrated in Fig. 1. After 2 h growth, a time corresponding to one cell division, the cytochrome aa_3 absorption at 605 mμ and cytochrome *b* absorption at 562 mμ are both reduced; in addition, new absorption bands at 587 and 508 mμ appear. The 587-mμ band probably corresponds to the so-called cytochrome a_1 which is thought to be a derivative of cytochrome aa_3. Cytochrome aa_3 absorption appears to be absent after 4 h growth. The absorption at 550 mμ characteristic of cytochrome *c* did not appear to diminish during the period of the experiment and after 16 h growth, when the cells had reached stationary phase, cytochrome *c* and perhaps a small amount of cytochrome *b* appeared to be the only contributors to the absorption spectrum. The same concentration of chloramphenicol had no apparent effect on the cytochromes of a control sample of yeast aerated for 16 h in 0.1 M KH_2PO_4. These yeast cells did not undergo division during the period of the experiment.

The decrease of cytochromes aa_3 and *b* in cells grown in the presence of chloramphenicol was paralleled by a drop in respiratory activity. Oxygen uptake of washed stationary phase cells was measured polarographically in the presence of excess glucose. Cells grown in the presence of chloramphenicol had a respiratory activity of less than 2 mμ atom O_2/mg dry wt. of cells per min, a value similar to that of anaerobically grown cells.

Normal aerobic cells have a respiratory activity of around 50 mμatom O_2/mg dry wt. of cells per min.

Electron micrographs of cells after 16 h growth in the presence of 4 mg/ml chloramphenicol have shown changes in the degree of organization of the mitochondria. Only a few diffuse mitochondrial profiles were visible, these structures did not appear to contain significant amounts of cristae. In addition, the chloramphenicol-inhibited cells contained noticeably more cytoplasmic membrane.

The foregoing observations on the growth, absorption spectrum, oxygen uptake and cytological appearance of *S. cerevisiae* cells grown in the presence of chloramphenicol have suggested to us that the antibiotic specifically inhibits the synthesis of some of the mitochondrial proteins. The normal growth of the yeast in excess fermentable substrate in the presence of a virtually saturated solution of chloramphenicol (4 mg/ml) would lead one seriously to question the idea that the drug has an inhibitory effect on cytoplasmic protein synthesis in all organisms. Chloramphenicol is known to arrest the growth of bacteria[8] as well as some more highly evolved organisms such as *Tetrahymena pyriformis* (ref. 1 and M. HUANG, D. R. BIGGS, G. D. CLARK-WALKER AND A. W. LINNANE, unpublished experiments) and *Euglena gracilis*[9,10]. However, in contrast to cell-free bacterial systems it does not inhibit the incorporation of amino acids by isolated cytoplasmic ribosomes from yeast[5,6], or from mammalian organisms[4]. The proposal offered by WEISBERGER AND WOLFE[11] to explain these apparent anomalies in terms of the rapidity of messenger RNA turnover appears to be unsatisfactory in the light of the present studies, as these authors' explanation rests on data confined to cytoplasmic ribosomal systems. We suggest it is more likely that chloramphenicol arrests the growth of higher organisms, dependent on intracellular organelles such as mitochondria and chloroplasts, because of a selective action of the antibiotic on these structures.

Furthermore, it appears as a generalization that the structural differences between bacterial ribosomes and ribosomes from the cytoplasm of higher organisms, reflected by their different sedimentation velocities, may be correlated with a functional difference in their sensitivity to chloramphenicol. In this regard ribosomes from chloroplasts resemble bacterial ribosomes in sedimentation velocity[12]. It is suggested that amino acid-incorporating systems of mitochondria in general may be more like those of bacteria than those from the cytoplasm of higher organisms.

This work was supported by the National Health and Medical Research Council of Australia and the U.S. Public Health Service (grant GM 10496-03). We are grateful to Parke Davis and Co. for the gift of chloramphenicol used in this study.

Biochemistry Department,
Monash University,
Clayton, Victoria,
(Australia)

MINTA HUANG
D. R. BIGGS
G. D. CLARK-WALKER
A. W. LINNANE

1 J. MAGER, *Biochim. Biophys. Acta*, 38 (1960) 150.
2 A. M. KROON, *Biochim. Biophys. Acta*, 72 (1963) 391.
3 E. WINTERSBERGER, *Biochem. Z.*, 341 (1965) 409.
4 G. VON EHRENSTEIN AND F. LIPMANN, *Proc. Natl. Acad. Sci. U.S.*, 47 (1961) 941.
5 A. G. SO AND E. W. DAVIE, *Biochemistry*, 2 (1963) 132.
6 R. K. BRETTHAUER, L. MARCUS, J. CHALOUPKA, H. O. HALVORSON AND R. M. BOCK, *Biochemistry*, 2 (1963) 1079.
7 P. G. WALLACE AND A. W. LINNANE, *Nature*, 201 (1964) 1191.
8 E. F. GALE, *Pharmacol. Rev.*, 15 (1963) 481.
9 R. M. SMILLIE, W. R. EVANS AND H. LYMAN, *Brookhaven Symp. Biol.*, 16 (1963) 89.
10 B. G. T. POGO AND A. O. POGO, *J. Protozool.*, 12 (1965) 96.
11 A. S. WEISBERGER AND S. WOLFE, *Fed. Proc.*, 23 (1964) 976.
12 M. L. PETERMANN, *The Physical and Chemical Properties of Ribosomes*, Elsevier, Amsterdam, 1964, p. 19.

Received October 18th, 1965

Biochim. Biophys. Acta, 114 (1966) 434–436