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synthesis by erythromycin.

Erythromycin has been reported to be an inhibitor of mitochondrial specific protein synthesis in yeast and the mutations conferring resistance to this antibiotic are cytoplasmically inherited. Such mutants possess mitochondria which are insensitive to the effects of erythromycin in a cell free protein synthesizing system. We have attempted to extend these observations to *N. crassa*, but have found that the effects of this antibiotic in *Neurospora* are significantly different from the effects found in yeast.

The inhibition of *Neurospora* growth at high concentrations of erythromycin (greater than 5 mg/ml) has been examined with regard to the effect of this antibiotic on in vivo protein synthesis. Conidia from wild type strain LSDT A (Harding et al. 1970 Arch. Biochem. Biophys. 138:653) were grown in Vogel's minimal medium with shaking at 30°C for four hours. The cells were collected by centrifugation, adjusted to a concentration of about 10⁶/ml in minimal sucrose medium, and incubated with varying concentrations of erythromycin for fifteen minutes. At the end of the incubation period, ¹⁴C-L-leucine (20 mC/mM) was added to a final concentration of 0.1 μC/ml and the cells were incubated another fifteen minutes at 30°C. The cells were collected on filter paper, washed five times with 5 ml each time 5% TCA made 0.1 M with respect to unlabeled leucine, and finally counted in Bray's solution. Under these conditions, 7100 cpm/ml were incorporated by cells in the absence of erythromycin, while duplicate samples containing 5 mg/ml erythromycin only incorporated 320 cpm/ml, which represents a 95% inhibition of leucine incorporation.

In addition, cells which had been incubated with ¹⁴C-L-leucine for 30 minutes, with and without erythromycin, were digested by treatment with Glusulase and disrupted by osmotic lysis. One ml aliquots were layered on a continuous 5-20% sucrose gradient and centrifuged for one hour at 25,000 rpm in a Beckman SW 50 rotor. Fractions of 0.5 ml were collected and assayed for incorporation of ¹⁴C leucine into hot TCA precipitable, NaOH soluble material. Under these conditions, it was found that the mitochondrial fractions from the erythromycin treated culture had incorporated only 37% as much leucine as that found in the mitochondrial fractions from the untreated culture. On the other hand, the cytosol fractions from the erythromycin treated culture contained only 3.4% as much labeled leucine as that found in the fractions from the untreated culture.

Taken together, these data suggest that erythromycin inhibits growth of *Neurospora* through inhibition of cytoplasmic protein synthesis, although it is still possible that mitochondrial protein synthesis is also inhibited. The fact that no mutants resistant to erythromycin have yet been obtained may indicate that more than one system is inhibited by this antibiotic. This work was supported by NIH grant # 12323 to R. P. Wagner. - - Department of Zoology, University of Texas, Austin, Texas 78712.