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A note on the inheritance of erythromycin-resistance in *Paramecium aurelia*

By G. H. BEALE
Institute of Animal Genetics, Edinburgh

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In yeast it has been reported that some types of erythromycin-resistance are due to cytoplasmic genetic factors, localized in mitochondria (Thomas & Wilkie, 1968; Slonimski, 1969). In view of the current interest in genetical aspects of mitochondria, and the ease with which cytoplasmic heredity can be demonstrated in *Paramecium aurelia*, some tests have been made to determine whether erythromycin-resistant variants could be obtained in this ciliate and, if so, to determine their genetic basis.

Paramecia were placed in bacterized lettuce medium at pH 7·0, to which erythromycin had been added to a concentration of 0·25 mg/ml. It was found that the organisms passed through two or three fissions, and then stopped dividing. Usually, however, they remained alive for days or weeks in presence of the drug, getting gradually smaller. To kill the paramecia outright, much higher concentrations of erythromycin were necessary. In the present study, however, higher concentrations of the drug than 0·25 mg/ml were not used.

Paramecia having the ability to grow in the presence of erythromycin were obtained in the following way. Samples of 10 ml, containing approximately 10000 organisms of *P. aurelia* (stocks 513 and 168, syngen 1), previously grown in bacterized lettuce medium, were put in centrifuge tubes, and gently spun down until most of the paramecia were at the bottom but alive. The supernatant was poured off and bacterized medium containing 0·25 mg/ml erythromycin added. The tubes were kept at 25 °C, and at twice-weekly intervals the contents again centrifuged and resuspended in freshly prepared bacterized medium with added erythromycin. After varying periods of time—between 2 and 4 weeks —some of the tubes were found to contain actively growing paramecia, and on isolating samples of these on to depression slides, the animals were found to grow at the normal rate (3 fissions per day at 25 °C) in erythromycin-containing medium. Once obtained, these clones were stable in regard to their resistance to erythromycin, for after being placed in normal bacterized lettuce medium and allowed to pass through several hundred fissions and several autogamies in the absence of the drug, the progeny were still found to be able to grow normally in erythromycin-containing medium.

To study the genetic basis of erythromycin-resistance in *P. aurelia*, conjugation was brought about between resistant animals of stock 513, and sensitives of stock 168. Pairs were isolated, and the cytoplasmic parentage of each ex-conjugant clone determined by testing one animal of each with specific antisera, after one post-conjugational fission (as described elsewhere, Beale, 1954). The sister animals were then tested for ability to grow in the presence of 0·25 mg/ml erythromycin. The F_1 progeny of 28 pairs were tested in this way, and in every case the exconjugant clones descended cytoplasmically from the erythromycin-resistant parent were found to consist of resistant animals, and the ex-conjugant clones descended cytoplasmically from the sensitive parent were all sensitive.

Backcrosses were made between erythromycin-resistant F_1 animals and stock 168 sensitives, and again one member of each pair was found to yield a clone of resistant

animals and the other a clone of sensitives. The same results were obtained after two further generations of backcrosses of the resistant progeny to the original sensitive stock. Since it is known that in *P. aurelia* there is normally little or no passage of cytoplasm between conjugants, but always a reciprocal exchange of nuclei, these results show that erythromycin-resistance in this material was due to a cytoplasmic factor. There was no evidence for the implication of nuclear genes.

Confirmation of this conclusion was obtained by studying the progeny of conjugating pairs, between which cytoplasmic exchange was artificially induced by mild serum treatment, as described by Sonneborn (1950). It was then found that *both* ex-conjugant clones from some pairs were resistant. In one case a clone of double animals was obtained by complete fusion of the conjugants, and this clone of doublets was also found to be resistant.

Some preliminary tests were made to investigate the mode of origin of the resistant lines. One thousand single isolates of stock 513 paramecia (not previously exposed to erythromycin) were individually isolated into bacterized medium containing 0·25 mg/ml of the drug. After 3 days at 25 °C one of the 1000 isolates was found to have given rise to a resistant clone, whilst the remainder had stopped dividing after three or fewer fissions. In another experiment, 12 10 ml cultures in erythromycin-containing medium were set up, with twice weekly resuspension in fresh medium, as described above. After 7–10 days, one of the 12 tubes was found to contain about 50 % resistant animals, while the remainder contained only sensitives, as judged from samples of 100 animals from each tube. After 14–18 days, two further tubes were found to contain 100 % resistant animals. Thus there appears to be a progressive increase of the cultures containing resistant animals, and a rapid over-running of the cultures by resistant animals once they have appeared. These experiments do not, however, answer the question whether erythromycin acts as a mutagenic agent inducing resistance to itself, or merely selects spontaneously occurring resistant cells, or intracellular components. Future work will be directed to elucidate this problem.

The present findings therefore show that in *P. aurelia*, as in yeast, ability to grow at the normal rate in the presence of a concentration of erythromycin which inhibits fission of normal cells is due to a cytoplasmic genetic factor. Whether or not this lies within the mitochondria is at present unknown.

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REFERENCES

BEALE, G. H. (1954). *The Genetics of* Paramecium aurelia. Cambridge.

SLONIMSKI, P. (1969). Mitochondrial DNA in yeast in relation to mutational or recombinational events. In *The Development and Interrelationships of Cell Organelles. Symp. Soc. exp. Biol.* (in the Press).

SONNEBORN, T. M. (1950). Methods in the general biology and genetics of *Paramecium aurelia*. *J. exp. Zool.* **113**, 87–143.

THOMAS, D. Y. & WILKIE, D. (1968). Inhibition of mitochondrial synthesis in yeast by erythromycin: cytoplasmic and nuclear factors controlling resistance. *Genet. Res., Camb.* **11**, 33–41.