# RNA AND PROTEIN SYNTHESIS IN AMACRONUCLEATE *PARAMECIUM AURELIA*

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Studies on anucleate cells have shown that in the absence of a nucleus there is appreciable protein synthesis (1, 5–7). Most of the anucleate cells used in these studies were produced by operative removal of the nucleus. Therefore, it seemed of interest to study these syntheses in a recessive mutant (*am*) of syngen 4 of *Paramecium aurelia* that produces amacronucleate cells by occasional failure of macronuclear division at cytokinesis (3, 4). Added interest is given to the problem because this species has been used extensively for studies of cytoplasmic inheritance. A comparison between paramecia containing and lacking the intracellular symbiont kappa was included to see if such symbionts would have any marked effect on the outcome.

TABLE I

*Grain Counts in Autoradiographs of Labeled Amacronucleate and Macronucleate P. aurelia*

| Kappa | Macronucleus | Grain counts per unit area above background | | | |
|---|---|---|---|---|---|
| | | Cytidine-uridine labeling | | | Amino acid labeling |
| | | No RNase | RNase | Difference | |
| None | Present | 533 ± 46 | 38.7 ± 4.0 | 494 ± 46 | 237 ± 15 |
| | Absent | 19.3 ± 2.5 | 10.4 ± 0.9 | 9.0 ± 2.7 | 73.9 ± 9.6 |
| | Ratio | 0.036 | 0.269 | 0.018 | 0.312 |
| None | Present | 241 ± 68 | — | — | — |
| | Absent | 6.5 ± 0.8 | — | — | — |
| | Ratio | 0.027 | — | — | — |
| Present | Present | 2000* | — | — | 501 ± 52 |
| | Absent | 39.9 ± 4.3 | — | — | 109.3 ± 7.6 |
| | Ratio | 0.020 | — | — | 0.218 |

* Estimated from sample counts on small areas. This is a minimum estimate since the number of grains was too high to count very accurately and overlapping probably occurred.

## MATERIALS AND METHODS

The paramecia were cultured in baked lettuce infusion with *Aerobacter aerogenes* as the food organism. Presumptive amacronucleates were selected on the basis of size and shape from log phase cultures, washed free of *Aerobacter*, and incubated for about 1 hour in sterile lettuce infusion containing either tritiated cytidine and uridine or a mixture of tritiated amino acids (10 μc each of tyrosine, isoleucine, proline, histidine, valine, phenylalanine, leucine, and lysine). A small amount of egg albumin was added to the mixture to promote uptake. Some presumptive macronucleate cells were included in each sample for comparison. At the end of the incubation, the cells were washed thoroughly in lettuce infusion, dried on slides, and fixed in ethanol:acetic acid (3:1). Cells incubated with the nucleosides were extracted with ice-cold 5 per cent (w/v) TCA for 5 minutes; those incubated with amino acids were extracted with 5 per cent TCA either at 25° or 90°C for 5 minutes. Some of the slides were digested with 1 mg/ml ribonuclease (RNase) for 3 hours at 25°C. The slides were washed free of TCA and covered with Kodak NTB3 liquid emulsion. Paramecia labeled with tritiated nucleosides were stained with methyl green–pyronin after the autoradiographs were developed; those labeled with tritiated amino acids were stained by the Feulgen procedure before covering with film. The autoradiographs were exposed before development for a time judged from test slides to be sufficient to produce

enough grains for convenient counting. All slides in a series were exposed for the same period. Grain counts were made over a unit area of cytoplasm as defined by a Whipple disk in the ocular. Twenty to 60 specimens of each group were counted.

## RESULTS AND DISCUSSION

The grain counts and their standard errors are shown in Table I. The amacronucleates incorporated about 2 to 3 per cent as much label from the nucleosides as did the macronucleates, and about 30 per cent as much label from the amino acids. The division at which the amacronucleates arose probably preceded labeling by several hours. The macronucleates may have been at any stage in the cell cycle. This should not have affected the comparison, however, since the counts per unit area of label incorporated from nucleosides and amino acids do not vary greatly during the cell cycle in this species (2, unpublished).

The results show that protein synthesis continues in the absence of the macronucleus with very little concomitant RNA synthesis. This agrees with the finding that protein synthesis occurs in other anucleate cells (1, 4–6). The lowered rate of protein synthesis may reflect the lack of production of new messenger RNA, but at least some messenger remains active for several hours since some of the amacronucleates must have been produced at division several hours before labeling.

The one point in which the findings do not agree entirely with some of the others is the existence of a small but statistically significant incorporation of label from nucleosides in the amacronucleates. Part of this incorporation remains after digestion with RNase; and, since the ratio between the amacronucleates and macronucleates is then nearly the same as with amino acid labeling, it may well be that some small part of the labeled nucleosides is diverted into protein synthesis. This still leaves a statistically significant RNase-removable incorporation, however. This incorporation might result from (*a*) RNA synthesis in the micronuclei which are present in most or all amacronucleates, (*b*) end-addition to preexisting RNA, (*c*) RNA synthesis by cytoplasmic DNA or cytoplasmic symbionts, if either exists in kappa-free material, or (*d*) DNA-independent RNA synthesis. The present experiments provide no basis for choice among these alternatives but show that such macronucleus-independent processes are not responsible for more than 3 per cent of the total RNA synthesis.

The experiments give no evidence for any contribution to RNA or protein synthesis by kappa. This is not too surprising since kappa, though it persists, is no longer able to multiply or transform into so called B particles in amacronucleates (4).

The Oak Ridge National Laboratory is operated by Union Carbide Corporation for the United States Atomic Energy Commission.

*Received for publication, January 14, 1964.*

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