THE BIOLOGICAL STATUS OF LAMBDA AND RELATED PARTICLES IN PARAMECIUM AURELIA

By W. J. van Wagtendonk, Jo Ann D. Clark, and G. A. Godoy

RESEARCH LABORATORIES OF THE VETERANS ADMINISTRATION HOSPITAL AND THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF MIAMI SCHOOL OF MEDICINE, CORAL GABLES, FLORIDA

Communicated by E. L. Tatum, September 26, 1963

The biological status of kappa and related particles has long been the subject of intensive investigations and many speculations.[1] Kappa has been variously looked upon as an endogenous genetic element, a virus, a rickettsia, a bacterium, or a degenerate alga. The evidence in support of the various viewpoints has been scant and mostly of a circumstantial nature. Sonneborn[2] has called these particles infectious, intracellular parasites, manifesting a protocellular level of organization, which would set them apart from typical bacteria, being either degenerate bacteria or intermediates between viruses and typical bacteria. Their size, sensitivity to antibiotics, reproduction by transverse division, and the presence of both DNA and RNA in the same particle would make them resemble rickettsiae or bacteria. On the other hand, the reported lack or paucity of enzymes and the failure to grow extracellularly would make them resemble viruses. The concentrations of DNA and RNA in the kappa[3] and lambda particles[4] are very similar and do not distinguish these particles from bacteria.

We wish to report here the successful *in vitro* cultivation of the lambda particle together with some of its biological properties.

Materials and Methods.—Axenically grown lambda-bearing *Paramecium aurelia*, stock 299, were obtained from Dr. A. T. Soldo (Biology Division, Schering Corporation, Bloomfield, New Jersey) and were grown in the medium described by him.[5] They were periodically checked for the presence of lambda particles using the aceto-orcein stain. A culture of the lambda-bearing paramecia (150 ml containing 10,000 organisms per ml) was concentrated by centrifugation at 1000 × *g*, the supernatant was decanted and the organisms in the remaining 5 ml were homogenized according to the method of Sonneborn.[6] The resulting homogenate was inoculated into 5 ml of various media contained in 15 × 200 mm culture tubes. All operations were carried out under the strictest aseptic conditions, and the inoculated tubes were always checked for foreign bacterial contamination.

One of the components of the media was a yeast fraction prepared as follows (Dr. A. T. Soldo, personal communication): The nondialyzable fraction from baker's yeast (strain 1821, Standard Brands, Inc.)[7] was prepared by stirring baker's dry yeast into glass-distilled water at 60°(1:4, w/v). The mixture was stirred for 2 hr at 60°. After autoclaving at 120° for 20 min the mixture was centrifuged and the clear supernatant poured into a section of cellulose dialysis tubing, immersed in distilled water with the open end above the water. After autoclaving, the open end was tied off and dialysis continued in running distilled water for 2 days. The impermeate was dried in the frozen state. The resulting dry powder was refluxed with methanol (1:100, w/v) for 30 min. The mixture was filtered and the extraction repeated once. The powder was washed once with acetone. This powder was slurried into water (50 mg per ml) and centrifuged at 25,000 × *g* for 15 min. The sediment was dried *in vacuo*. According to Allfrey and King,[8] folic acid should have been removed completely from this preparation. It contains carbohydrate and protein but no nucleic acid or lipids. The treatment precludes the presence of nondenatured proteins or small peptides.

Tests for killing ability were carried out using *P. aurelia* of stock 92, syngen 3, grown in a monoxenic medium (baked lettuce infusion inoculated with *Aerobacter aerogenes*) as testers. Two hundred to 300 sensitive organisms, contained in a volume of approximately 0.1 ml, were placed

in wells of a depression slide and serial dilutions of the lambda particles were added. The depressions were observed for 90 min and the number of killed and lysed sensitives was recorded.

Results.—Of the various media tried, originally only the medium reported in Table 1 would support the growth of the lambda particles. The particles multiply at the rate of about one fission per day. Cultures of the lambda particles have now been maintained for 24 weeks, with transfers made every week. It has since been

TABLE 1

COMPOSITION OF THE MEDIUM FOR THE GROWTH OF THE LAMBDA PARTICLES

	Conc.*		Conc.
Glycine	40	Na EDTA	500
L-Alanine	60	Adenosine	500
DL-Serine	60	Edamine S	5 mg
DL-Threonine	70	Glycerol	10 mg
DL-Valine	40	Maltose	2.5 mg
DL-Leucine	50		
DL-Isoleucine	50		
DL-Methionine	60	Ca Pantothenate	5
DL-Asparagine	36	Nicotine amide	5
L-Glutamic acid	80	Pyridoxal H Cl	5
L-Aspartic acid	20	Pyridoxamine H Cl	2.5
L-Lysine H Cl	50	Riboflavin	5
L-Arginine H Cl	60	Folic acid	2.5
DL-Histidine H Cl	40	Thiamine H Cl	15
L-Phenylalanine	40	Biotin	1.25 m μg
L-Tyrosine	15	α-Lipoic acid	0.05
L-Tryptophan	30	Stigmasterol	0.4
L-Proline	40	Yeast fraction	500
$MgSO_4$	250	0.001 *M* Na phosphate pH 6.8	20 ml/100 ml
Guanosine-mono-phosphate	250		
Uridine-mono-phosphate	250		

* All concentrations are in μg/ml, unless otherwise specified.

found that exogenous folic acid is not required, thereby confirming the observation of Soldo[9] that the lambda particles can synthesize folic acid.

The observation of Soldo that lambda particles are gram-negative rod or diplorod type structures[9] was confirmed with the *in vitro* grown particles (Fig. 1). The particles have retained their ability to kill sensitive paramecia, as can be seen from Table 2. There is a fair degree of proportionality between the number of particles present and the number of sensitives killed. If axenically grown sensitives are used, the killing action of comparable numbers of lambda particles is about 60 per cent less.

TABLE 2

THE KILLING ACTION BY INCREASING AMOUNTS OF LAMBDA PARTICLES

No. of λ particles	No. sensitives killed
6	0
30	3
60	9
120	21

Lambda particles grown *in vitro* were tested for their ability to infect cells of stock 299, which had become sensitives through the loss of the particles. An axenic stock of 299-sensitive cells was cultured in a medium containing 100 units of penicillin per ml for 4 days in order to ensure that the animals were lambda-free.[9] They were then transferred to the regular axenic medium. One hundred to 200 sensitive paramecia contained in 0.2 ml of culture medium were placed in the well of a sterile depression slide. To this were added 0.02 ml of a suspension of lambda particles containing approximately 400 particles. The depression slides were placed in a

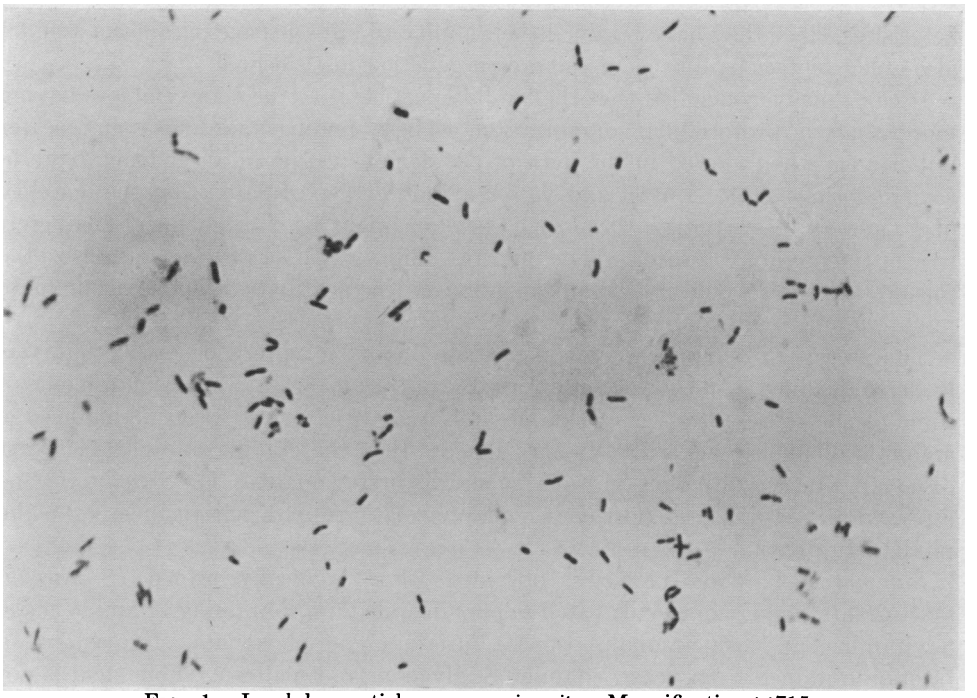

FIG. 1.—Lambda particles grown *in vitro*. Magnification ×715.

moist chamber and left overnight. The next day the surviving animals (about 50% of the original) were transferred to tubes containing the axenic medium and allowed to grow up. At the end of 5 days these animals were transferred to a lettuce infusion inoculated with *A. aerogenes*. In this bacterized state the animals were tested for their killing ability in the usual manner. Blistering and lysis of sensitive animals were observed within 30 min. The infected animals were also stained with aceto-orcein, and the presence of the lambda particles in the cytoplasm was ascertained.

Discussion.—The above findings necessitate a re-evaluation of the biological status of kappa and its related particles. These particles are clearly endosymbiotes, which can also be grown *in vitro*. Various reports in the literature emphasize the postulated fact that these particles demonstrate a lack, or at least a paucity, of enzymes. This assumption is invalid. First, Soldo[9] has demonstrated that the presence of the lambda particles eliminates the requirement of folic acid for stock 299 of *P. aurelia*. Since the lambda particle can also grow in an axenic medium in the absence of folic acid, it therefore possesses the elaborate enzymatic machinery for the synthesis of folic acid. Second, the ability of the lambda particle to grow *in vitro* indicates that the particle must be able to synthesize its cellular components from the constituents of the medium. The lambda particle requires, as does *P. aurelia*, a factor (or factors) present in the yeast fraction. Thus far, attempts to replace this fraction with known substances have been unsuccessful. The original homogenate is diluted out in serial transfer and thus is not contributing other unknown nutrilites to the medium.

The *in vitro* culture of the lambda particles has not changed their biological char-

acteristics, since they have retained their ability to kill sensitive paramecia and are also able to infect paramecia of the proper genetic constitution.

It can thus be concluded that the lambda particle is a true endosymbiote having more than a protocellular organization. These findings assume even greater importance when viewed in the light of the demonstration of an endosymbiote in *Crithidia oncopelti*.[10] Butzel and van Wagtendonk[11] postulated that the inability of *P. aurelia* to synthesize the purine skeleton might be a biochemical characteristic of the phylum Protozoa. The reported ability of *C. oncopelti* to synthesize the purine skeleton[12] would not be an exception to this postulate. The endosymbiote might well be responsible for the synthesis of the purines.

The presence of endosymbiotes in protists might be more widespread than was hitherto assumed, and thus account for some of the atypical growth requirements of some of the representatives of this phylum and their ability to accumulate large amounts of nucleic acid.[13,14] It also appears that the cytoplasmic particles first discovered in *P. aurelia* are not unique as was first thought. They might well be representatives of a large host-endosymbiote relationship of various degrees in the phylum Protozoa.

Summary.—Lambda particles, obtained from axenically grown *P. aurelia*, stock 299 (killers), can be cultivated *in vitro* in a medium containing 17 amino acids, 8 vitamins, guanosine-mono-phosphate, uridine-mono-phosphate, stigmasterol, a fraction obtained from yeast, edamine S, glycerol, and maltose. Folic acid is not required. The *in vitro* grown particles have retained their killing ability and can, under appropriate conditions, infect sensitive *P. aurelia* of stock 299. It is concluded that these particles are bacterial endosymbiotes of *P. aurelia*, stock 299.

Supported by a grant from the National Institutes of Health (E-3644). One of us (J. A. D. C.) held a National Defense Act fellowship during part of this study.

[1] Sonneborn, T. M., *Advan. Virus Res.*, **6**, 229 (1959).

[2] Sonneborn, T. M., in *Perspectives in Virology*, ed. M. Pollard (Minneapolis: Burgess Publishing Co., 1961), vol. 2, p. 5.

[3] Smith, J. E., and W. J. van Wagtendonk, *Fed. Proc.*, **21**, no. 2, 153 (1962).

[4] Tanguay, R. B., and W. J. van Wagtendonk, *Fed. Proc.*, **22**, no. 2, 646 (1963).

[5] Soldo, A. T., *Proc. Soc. Exptl. Biol. Med.*, **105**, 612 (1960).

[6] Sonneborn, T. M., *J. Exptl. Zool.*, **113**, 87 (1950).

[7] Miller, C. A., and W. J. van Wagtendonk, *J. Gen. Microbiol.*, **15**, 280 (1956).

[8] Allfrey, V. G., and C. G. King, *J. Biol. Chem.*, **182**, 367 (1950).

[9] Soldo, A. T., *Ann. N. Y. Acad. Sci.*, **108**, 380 (1963).

[10] Gill, J. W., and H. J. Vogel, *J. Protozool.*, **10**, 148 (1963).

[11] Butzel, H. M., Jr., and W. J. van Wagtendonk, *J. Gen. Microbiol.*, **30**, 503 (1963).

[12] Nathan, H. A., *J. Protozool.*, **5**, 194 (1958).

[13] Newton, B. A., *J. Gen. Microbiol.*, **17**, 708 (1957).

[14] Johnson, B. F., and T. W. James, *Exptl. Cell Res.*, **20**, 66 (1960).