THE RELATIONSHIP BETWEEN KAPPA AND PARAMECIN IN PARAMECIUM AURELIA*

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Three types of genetic cytoplasmic particles, similar in number per animal and staining reactions, have been described in *Paramecium aurelia*. They are designated by the Greek symbols kappa, mu, and pi, and are characterized as follows.

Kappa particles are distinguished by the fact that animals containing them are "killers," for they produce a toxin called "paramecin." This toxin causes death to "sensitive" paramecia. Both the culture medium and breis from killer animals contain paramecin. Kappa was at first described and studied only by genetic techniques[1, 2] and it was not until later that cytological techniques[3] identified kappa with microscopically visible Feulgen-positive bodies. Each killer strain produces a characteristic paramecin (distinguished by the prelethal responses elicited from sensitives) and mutations of kappa have been recognized by changes in paramecin production.[4] Although much data concerned with the genetic, physiological, and chemical nature of kappa and paramecin are available,[5] the relationship between the two has remained obscure.

Mu particles are characterized by the fact that animals containing them are "mate-killers" and cause the death of their sensitive mates following conjugation.[6] Although mate-killers may produce toxins, these are unlike paramecin in that they are not detectable either in breis of mate-killers or in the culture fluid in which mate-killers have lived.[7]

Pi bodies are distinguished by the fact that they are not associated with any demonstrable lethal effect. They were first found cytologically by Hanson[8] in a strain which had once been a killer, and are probably kappa mutants. Of chief interest here is the fact that neither mu nor pi bodies are associated with paramecin production.

Recent studies[9] with the phase microscope revealed that kappa bodies from any killer paramecium are of two major types: "brights" and "non-brights." Brights contain one or two characteristic refractile regions while non-brights do not. Our first clue as to the identity of paramecin was provided by a comparative study of kappa, mu, and pi bodies. Brights occur only in animals producing paramecin. The present communication presents this and other evidence indicating that brights are the bearers of paramecin activity.

Materials.—The following strains of *P. aurelia* were used. Killer stocks G, H, 36, and 50 belong to variety 2 and have been previously described.[2]

Gm1 is a mutant killer derived from stock G.[2] Killer stocks 47,[10] 51,[10] 116,[11], and 169[11] belong to variety 4.

Stocks 130, 131, and 138 belong to variety 8 and are mate-killers.[11]

Dippell[4] has isolated mutant killers from stock 51 and shown that they contain mutant kappas. We have used two strains, 51ml and 51m5, previously described by her, and two, "derived" 51ml and "derived" 51m5, which she has isolated more recently from other mutants (Dippell, personal communication). When these four strains were tested for killing in this laboratory it was found that three of them (51ml, 51m5, and "derived" 51m5) had lost the ability to kill. However, cytological examination revealed that these contain kappa-like cytoplasmic bodies. These bodies thus appear to be similar to the pi bodies discovered by Hanson. They probably represent kappa mutants, each having arisen independently from the kappa in Dippell's strains after they were first isolated by her as mutant killers.

A strain of *P. caudatum*, stock 79a,[3] was used to test the paramecin activity of stock G.

Methods.—The standard methods described[12] for the study of *P. aurelia* were used. Cytoplasmic particles were observed in unfixed material with the phase-contrast microscope after the paramecia had been crushed.[9] The preparation of stained material will be described below.

To compare the mobility of paramecin with kappa and trichocysts in centrifugal fields, breis of killers were centrifuged. To prepare such breis, stock G killers were filtered through 16 layers of cheesecloth and concentrated by centrifugation. Most of the bacteria in the concentrate were removed by suspending and recentrifuging the cells three times in an excess of 0.25% sterile yeast extract. To permit the digestion of many of the bacteria present in food vacuoles, the paramecia were allowed to remain for one to two hours at 27°C. in the yeast medium. The animals were suspended and concentrated as before, transferred to 10% amphibian Ringer's solution, and homogenized with the aid of a syringe. Centrifuge tubes were made from glass tubing (with an inside diameter of 5 mm.), one end of which was fused. One-ml. samples of the brei containing approximately 5×10^4 broken paramecia were centrifuged in a horizontal centrifuge for ten minutes at various speeds. Then the top 0.9 ml. of each brei, the "supernatants," were carefully removed from the tubes leaving as the "precipitates" the bottom 0.1 ml. The amount of paramecin in the supernates and precipitates was determined by recording the number of affected sensitives in serial dilutions (made in 10% Ringer's). That dilution which affected about 25 of 200 stock 79a sensitives after 24 hours at 27°C. was considered the end-point. From a comparison of the end-points of the supernate and precipitate at a given centrifuge speed, the "per cent down" or the amount of paramecin which moved from the top 0.9 ml. to the bottom

0.1 ml. of the centrifuge tube was calculated. There was no evidence of paramecin inactivation due to centrifugation. As a control, breis from sensitives of stock G (these produce no paramecin) were prepared and centrifuged as described above. No killing activity was detected among the controls.

To determine the mobility of kappa and trichocysts, 0.0025-ml. samples of supernate and precipitate (or suitable dilutions of them) were carefully spread on 1 cm.^{2} areas of glass slides. The preparations were air dried, stained for one minute in 0.25% aqueous gentian violet, and examined under oil immersion. The stained kappa bodies and trichocysts in 10 to 40 microscope fields were counted and the average number per field was used to calculate the per cent down. As before, the centrifugation did not reduce

TABLE 1

THE OCCURRENCE OF "BRIGHTS" AMONG STRAINS OF *P. aurelia* CONTAINING KAPPA, MU, AND PI

VARIETY	STRAIN	RESPONSE OF SENSITIVES	CYTOPLASMIC PARTICLES DESIGNATION	BRIGHTS
2	G	Spinning	Kappa	Present
	Gm1	Paralysis	Kappa	Present
	H	Vacuolization	Kappa	Present
	36	Spinning and vacuolization	Kappa	Present
	50	Spinning and vacuolization	Kappa	Present
4	47	Humping	Kappa	Present
	51	Humping	Kappa	Present
	51m1	None	Pi	Absent
	De 51m1	Spinning	Kappa	Present
	51m5	None	Pi	Absent
	De 51m5	None	Pi	Absent
	116	Humping	Kappa	Present
	169	Humping	Kappa	Present
8	130	Mate-killing	Mu	Absent
	131	Mate-killing	Mu	Absent
	138	Mate-killing	Mu	Absent

the total numbers of particles. Stained samples of control breis (prepared from stock G sensitives) contained trichocysts but did not reveal significant numbers of stained bodies which could be confused with kappa.

Results.—Samples of the stocks described above and their mutants were maintained at 0.2 to 0.5 fission per day at 27°C. for two to eight weeks and then the particles present in each were observed with the phase-contrast microscope. Paramecia from each of the ten killer strains were always found to contain brights, the per cent brights among all particles varying from 4 to 30%. Paramecia from the six strains containing mu or pi bodies have never contained brights. These data are summarized in table 1. Hanson's[8] finding that two pi-bearing cultures of independent origin are free of brights confirms and extends these observations.

These results led to the hypothesis that the particle with paramecin activity is the bright particle. A test of the hypothesis was suggested by the preliminary experiments of the senior author which indicated that the centrifugal mobility of brights was very similar to that of paramecin. Consequently, a careful comparison of the mobility of paramecin, brights, and total stainable bodies was undertaken. Stock G was selected as a representative killer because its brights are easily distinguished from non-brights and from bacteria in stained breis. Killer breis were prepared, centrifuged, and assayed as described above.

The mobility of paramecin is graphically represented in figure 1. The smooth curve was obtained by fitting a curve of the form $Y = 1 - e^{-ax}$ to the data by least squares. This equation has no theoretical significance and was chosen only because it was found empirically to fit the data. The points for the sum of brights and non-brights were found to lie well below the curve for paramecin as can be seen in figure 2. But when the brights alone were scored, it was found that the resulting points (also shown in figure 2) approximated very well the paramecin curve.

An attempt was made to study the sedimentation rates of other particulates present in breis. This was done in the case of trichocysts. Undischarged trichocysts were more mobile and discharged trichocysts were less mobile than paramecin.

Discussion.—The presence of brights in ten strains which produce paramecin and the absence of brights in eight strains which do not produce paramecin, clearly indicates a relationship between brights and paramecin. The extremely good agreement between the data for brights and paramecin in respect to centrifugal mobility leaves little doubt that the bright particle is the particulate in breis which carries paramecin activity.

This conclusion is in agreement with the findings that paramecin and brights both contain desoxyribose nucleic acid[3, 9, 13] and that paramecin particles in breis are never more numerous than brights.[14]

Nanney[15] has studied the relationship between kappa and paramecin using x-radiation, and has reached conclusions compatible with ours. His data indicate that, although the two are not identical, kappa and paramecin activities could be associated with the same particle.

It has been shown that the number of genetic kappa particles is approximately equal to the number of visible cytoplasmic bodies in a killer and these bodies have been established as kappa.[3] The interpretation of these numerical data is not quite so clear when one realizes that the cytologically visible bodies actually are made up of two categories—brights and non-brights. If the agreement in the numbers of kappa particles and visible bodies were sufficiently precise, one would have to conclude that brights and non-brights are both kappa. But since brights are present in rather low frequency, one can only be sure that non-brights represent kappa par-

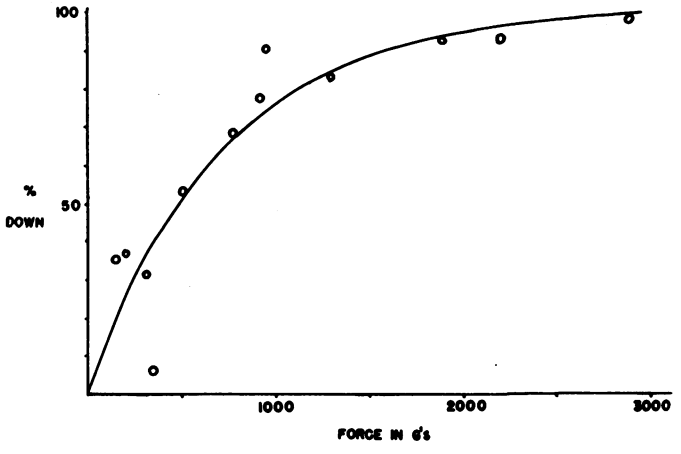

FIGURE 1

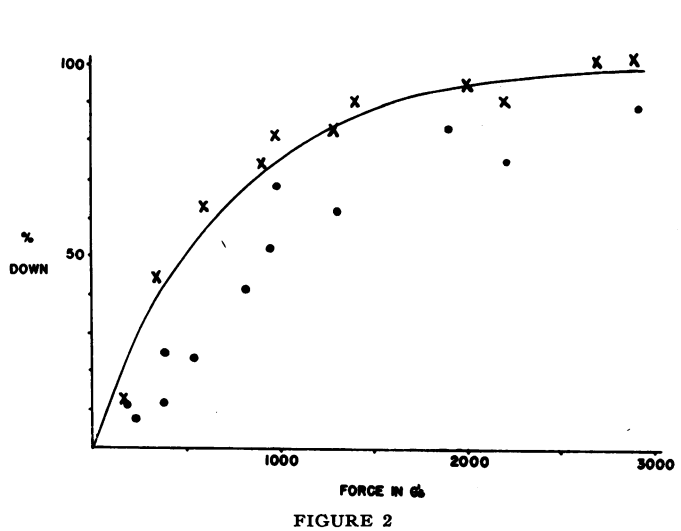

FIGURE 2

Figs. 1 and 2. Comparison of the sedimentation rates of paramecin, kappa, and brights. The per cent particles centrifuged down by different centrifugal forces is plotted.

In figure 1, paramecin is represented by open circles. The smooth curve is of the form $Y = 1 - e^{-ax}$ and was fitted to the points by least squares. In figure 2, kappa (brights plus non-brights) is represented by solid circles and brights are represented by x's. The smooth curve represents paramecin and is the same as the one shown in figure 1.

ticles. If paramecin particles are brights, and since kappa is responsible for the production of paramecin, it follows that non-brights must be responsible for the production of brights.

It is thus clear that mutations of kappa to pi involve a loss of the capacity to *produce* brights. Similarly, mu bodies might represent mutant kappas, although the possibility of an independent origin cannot be excluded.

The question of whether brights are capable of producing new particles has not been answered. Double non-brights have been interpreted as stages in the division of non-brights.[9] If brights can produce new particles then one might expect to find brights which are double in appearance. Actually they are found occasionally, one of the two portions having a refractile area, the other usually without the refractile area.

Attempts to stain the refractile bodies in brights have been unsuccessful and the chemical nature of the structure is unknown. It has been suggested that if kappa is a bacterial symbiont, the refractile bodies may be endospores, but it is noted that bacterial spore stains are negative.[9] Delamater (personal communication) and Sonneborn (unpublished) suggested the possibility that the refractile body may be a virus or viral inclusion body, but there is no pertinent evidence at this time.

Summary.—A small proportion of the cytoplasmic bodies which have been identified with kappa in *P. aurelia* contain one or more refractile regions. Bodies with these regions are called brights. Only those strains which produce the toxin paramecin contain brights. Although animals of other strains unable to produce paramecin may contain kappa-like cytoplasmic bodies, they lack brights. The sedimentation rates of paramecin and brights are indistinguishable, while the sedimentation rate of non-bright kappa is different. It is concluded that brights are the bearers of paramecin activity and that they are produced by non-bright kappas.

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