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SHORT PAPERS

Some additional results on the maintenance of kappa particles in *Paramecium aurelia* (stock 51) after loss of the gene *K*

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Although it was shown by Sonneborn (1943) that the cytoplasmic kappa particles of *Paramecium aurelia* could be permanently maintained only when the paramecia contained the gene *K*, later work of Chao (1953) and Yeung (1965) showed that there was often a surprisingly long delay between loss of the gene *K* and disappearance of the kappa particles. As regards the exact stage at which the particles vanished, much variation has been found, and this seemed to be quite unlike the remarkable regularity with which cytoplasmic particles of another type (mu) were reported to disappear from cells deprived of the genes *M1* and *M2* (Gibson & Beale, 1962). Based on the latter results, the 'metagon' theory was put forward, though the present status of this theory is uncertain since we have repeatedly failed to reproduce the conditions under which regular loss of mu particles occurs.

In spite of these irregularities there is no doubt that under some conditions kappa and mu particles continue to maintain themselves for many cell generations of *Paramecium*, even in the absence of the appropriate genes. In view of the importance of trying to find out the nature of the underlying factor or factors controlling the growth of these particles, we have made a further study of the maintenance of kappa particles in clones recently deprived of the gene *K*, and paid particular attention to the variability in the stage at which the kappa particles disappear.

The material and methods used here were the same as those described by Yeung (1965), except that kappa particles were here identified by staining and microscopical observation by the method of Beale & Jurand (1966).

Experiment 1. To indicate the great variability in rate of loss of kappa particles, we give in Table 1 some results showing the proportions of paramecia with and without particles after ten fissions following loss of gene *K*. Each group of paramecia forms a clone obtained as follows. Animals of genotype *Kk*, in autogamy, and containing kappa particles, were allowed to divide once, and one of the first fission products from each pair was used to identify the new, post-autogamous genotype (*KK* or *kk*). The other 'first fission' animal, if *kk*, was then allowed to grow to the 'tenth fission' stage, in the following manner. The 'first fission' animal was allowed to divide three times, yielding eight animals, which were re-isolated and allowed to divide a further three times. The sixty-four animals thus obtained were re-isolated and allowed to divide a further three times, which would (ideally) give rise to sixty-four groups each containing eight animals at the 'tenth fission' stage. Single animals from each of the 'tenth fission' groups were stained and examined for presence of kappa particles. In practice, however, fewer than sixty-four groups of tenth fission animals were usually available, due to dying out of some sub-lines, or to loss

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on handling. Only animals derived from cultures which had grown optimally throughout the whole period up to the tenth fission were used in the experiment, so that the number of fissions undergone was known with certainty.

Table 1. *Numbers of paramecia with and without kappa particles after ten fissions following loss of gene* K

Clone No.	Kappa present	Kappa absent
1	34	0
2	44	0
3	36	0
4	34	7
5	57	3
6	13	25
7	50	2
8	63	0
9	43	0
10	0	46
11	14	30
12	45	0
13	36	7
14	11	43
15	16	40
16	19	37
17	0	44
18	22	28
19	11	39
20	2	16
21	0	37
22	19	26
23	4	48
24	36	24

It appears from Table 1 that there is great inter-clonal variation in the stage at which kappa particles disappear. To investigate this further, a second experiment was performed, in which a small number of clones and sub-clones were studied in detail for as long as the kappa particles could be maintained.

Experiment 2. Three clones (Nos. 25, 26, 27) were taken and samples stained at the eighth and again at about the eleventh fission stages (see Table 2). Six animals were then chosen from each of these '11th fission' groups, and grown for about three more fissions, after which all but one of the animals from each group were stained. Where most or all of the stained animals still contained particles, the remaining one in each group was grown on further, and the process repeated until no animals containing particles remained. In view of the number of cultures under observation simultaneously and the lack of synchrony in fission, it was not possible in this experiment to specify the exact number of fissions undergone by the tested animals.

The results of this experiment are set out in Table 2. From this it can be seen, firstly, that in some animals kappa particles were still present after as many as twenty-six or twenty-seven fissions following loss of gene *K*. Confirmation that the genotype (at least of the micronuclei) of these clones was in fact *kk* was obtained by further test-matings to *KK* animals and the observation of a 1:1 segregation of animals with and without particles after a subsequent autogamy.

Secondly, the data in Table 2 show that the fluctuation in stage at which loss of kappa particles occurs is not a clonal characteristic. Thus in clone 27 some animals lost kappa

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Table 2. *Detailed study of clones and sub-clones still containing kappa particles after fifteen fissions*

	8 Fissions		10/12 Fissions		15/16 Fissions			20/21 Fissions			22/23 Fissions			24/25 Fissions			26/27 Fissions			30 Fissions		
Clone	Kappa present	Kappa absent	Kappa present	Kappa absent	Sub-clone	Kappa present	Kappa absent	Sub-clone	Kappa present	Kappa absent	Sub-clone	Kappa present	Kappa absent	Sub-clone	Kappa present	Kappa absent	Sub-clone	Kappa present	Kappa absent	Sub-clone	Kappa present	Kappa absent
25	53	0	20	9	(1)	0	all*															
					(2)	all*																
					(3)	all*	0	(3a)	28	0	(3a)	1	1									
								(3b)	26	0	(3b)	3	0	(3b)	0	3						
					(4)	0	all*															
					(5)	all*	0	(5)	18	5	(5)	1	6									
26	60	3	13	21	(1)	0	all*															
					(2)	0	all*															
					(3)	0	all*															
					(4)	0	all*															
					(5)	0	all*															
					(6)	all*	0	(6a)	16	8												
								(6b)	26	1				(6b)	7	0	(6b)	7	0	(6b)	0	9
								(6c)	13	11												
27	29	20	7	19	(1)	0	all*															
					(2)	10	3	(2)	11	3	(2)	0	3									
					(3)	all*	0	(3a)	0	10												
								(3b)	25	0	(3b)	7	0							(3b)	0	13
					(4)	8	8	(4)	0	22												
					(5)	all**	0	(5)	14	0	(5)	2	0	(5)	3	0				(5)	0	11
					(6)	all**	0	(6)	24	2	(6)	7	0	(6)	0	3						

Notes

* 'all'—signifies that all except one of the available animals were stained and found to contain kappa particles. The exact numbers varied, but usually there were between eight and sixteen, of which all except one were stained and the remaining one carried on, if particles were present.

** —in these cultures occasional animals lacked particles, but most had them.

particles before the eighth fission whilst others maintained them for twenty-four or twenty-five fissions. Animals in a given clone of paramecia may suddenly lose the particles at any time over this period.

Thirdly the data in Table 2 confirm the finding of Yeung (1965) that an animal containing kappa particles can give rise to a group of progeny none of which contain particles. This is contrary to the metagon theory of Gibson & Beale (1962) according to which the progeny of any paramecium containing (mu) particles must include at least one animal containing particles.

In spite of the apparently unpredictable character of the change resulting in loss of particles, some conditions are known to increase the probability that such loss takes place, at least in regard to kappa particles. Yeung (1965) showed that starvation had such an effect. In the present study, evidence has accumulated that when the growth rate was somewhat less than the maximum expected under the given environmental conditions, loss of kappa particles was very likely to occur. This was therefore investigated further in Experiment 3.

Experiment 3. A series of ex-autogamous clones (genotype *kk*, initially with kappa) was prepared as before, and all the animals were classified in regard to possession of kappa particles after ten fissions. Care was taken to ensure that the number of fissions undergone was in fact exactly ten, by the procedure described in Experiment 1. All these animals were supplied continuously with excess bacterized lettuce medium. Some clones were grown throughout at 18° C., others were grown at first at 18° C. and later transferred to

Table 3. *Correlation between fission rate of ex-autogamous* kk *animals and presence of kappa particles after ten fissions. Growth was at 18° C. except as indicated*

Clone	Days between autogamy and attainment of ten fissions	Nos. of paramecia containing kappa	Nos. of paramecia lacking kappa
28	5	34	0
	6	2	7
29	6	14	30
	7	0	4
30	4	16	0
	5	3	30
	7	0	7
31	5	20	11
	5 (+5 hr. at 27°C.)	0	4
	6	2	9
	7	0	4
32	5	11	14
	5 (+5 hr. at 27°C.)	0	13
	6	0	12
33*	3	2	0
	4	0	2
	5	0	14
34*	3	19	14
	4	0	10
	5	0	2
35*	3	34	15
	4	2	7
	5	0	2

These three clones were grown at 18°C. for 2 days (about five fissions) and then at 27°C.

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27° C., as indicated in Table 3. It was found that, even within the same clone, some animals reached the tenth fission earlier than others. The cause of this variation is unknown, since the animals within a clone were identical in gene constitution and were exposed to the same environmental conditions. In Table 3 the results are set out to show the number of days required to attain ten post-autogamous fissions, and the numbers of animals with kappa particles. It is clear that a slight retardation in fission rate is correlated with a dramatic loss of kappa particles. From this one cannot conclude, however, that the retardation is the cause of the particle loss: the two phenomena might have a common cause.

The interest of these results lies in the information they provide towards helping us understand the mechanism whereby a gene (*K*) of *Paramecium* enables the cell to maintain a population of cytoplasmic symbionts. According to the metagon theory as originally proposed by Gibson & Beale (1962), this mechanism involved the liberation into the cytoplasm of stable, non-replicating gene products or metagons. The present results indicate first that the agent supporting kappa particles may be *unstable*, especially under conditions producing sub-optimal growth, and secondly, the long-continued maintenance of kappa in some branches of a clone, supports the notion of a *replicating* determinant. Thus these results certainly do not support the metagon theory as previously proposed. If this theory is to be maintained any longer we shall have to postulate the existence of accessory factors which (*a*) cause the metagon to become unstable, and (*b*) cause it to replicate.

At the present stage, it does not seem profitable to speculate further.

SUMMARY

Additional data are presented on the maintenance of kappa particles in cells of *P. aurelia* (stock 51) after removal of the gene *K*. It is shown that kappa particles may be maintained for as long as twenty-seven fissions in the absence of *K*, or may disappear before eight fissions. Slight retardation in fission rate of paramecia quickly eliminates kappa particles. The bearing of these results on the metagon theory is discussed.

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Twin-spotting attributed to somatic crossing-over in a mouse of constitution $aac^{ch}c$ (fawn). The contrasting patches would be $a\ a\ c^{ch}\ c^{ch}$ (dark brown) and $a\ a\ c\ c$ (white).

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