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THE ROLE OF SYMBIONTS AND AUTOCATALYSTS IN THE GENETICS OF THE CILIATES

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ONE of the things that distinguish Paramecium from the general run of cells is its enormous size. In fact Paramecium is large enough to be easily visible against a dark background with the naked eye. This enormous size is of advantage to Paramecium in the capturing of its food and in protecting it against attack by smaller organisms. But it was also of advantage, in the course of evolution, for Paramecium to retain the rapid rate of reproduction and dispersal made possible by a unicellular organization. The Protozoa in general are larger than the average cell for the same reason.

There are certain peculiar features in the heredity and development of Paramecium connected with its enormous size. In the first place, the large cell size has led to the evolution of a correspondingly large nucleus. For in all cells there is a definite size relationship between nucleus and cytoplasm. Increase in cell size necessitates a corresponding increase in nuclear size; the reverse is also true as in polyploids and in the salivary gland cells of Drosophila. In Paramecium, increase in nuclear size came about by the formation of the macronucleus, a compound body consisting of about a hundred diploid nuclei each still enclosed in its own membrane. At cell division, it is probable that each diploid nucleus divides mitotically (forming about two hundred diploid nuclei, one hundred for each new Paramecium if this was the original number). The macronucleus as a whole then divides by "amitosis," though the individual nuclei do not. (In Protozoa in general the nuclear membrane does not disappear at mitosis; this probably applies to the membranes of the individual diploid nuclei in the macronucleus of Paramecium.)

By virtue of its size, the macronucleus would be expected to control the physiological processes of the cell, to the exclusion of the micronucleus; for at each locus it would contain a hundred pairs of genes (if the number of its diploid nuclei is one hundred), and these hundred pairs would be dominant to the single pair in the micronucleus of *P. caudatum* or even to the two pairs in the double micronucleus of *P. aurelia*. This would be true even if the genes in the macronucleus were individually recessive and at least one of those in the micronucleus dominant (a situation which is experimentally possible by regeneration of the old macronucleus pure for a recessive gene in an ex-conjugant heterozygous for the dominant allele received from the other parent). But because of its highly compound nature the macronucleus cannot very well undergo the orderly process of meiosis and Mendelian segregation, and so must undergo degeneration at meiosis if sexual reproduction is to be effective. Hence an ordinary diploid nucleus—one capable of meiosis—becomes necessary in addition and is represented by the micronucleus.

But the two kinds of nuclei (macro- and micro-) almost certainly do not differ in the fundamental character of their individual genes—the one does not produce plasmagenes if the other does not. Neither does the macronucleus differ fundamentally from the ordinary nuclei of other organisms, except for its compound nature and resultant larger size—the thing that makes it the physiological (or somatic) nucleus of Paramecium—and this we saw was merely a result of increased cell size. It is true that the macronucleus degenerates at the time of sexual reproduction, but this is somewhat comparable to the degeneration of the nucleus in the case of the red blood corpuscles, made necessary on physiological grounds. Perhaps it comes about in the case of Paramecium through the action of a nuclease with a very localized distribution. In any event, the new genotype produced by conjugation could not express itself unless a

new macronucleus replaced the old one.

The large cell size of Paramecium led to a second complication. Size made possible a degree of differentiation not found in single-celled organisms of smaller size. Thus the cytoplasm came to serve as the equivalent of the soma of multicellular organisms. But it also had to serve as material for the germ cells at the time of conjugation. Hence it became necessary that the cytoplasm of Paramecium return at the beginning of each sexual cycle to the undifferentiated state usual for germ cells. The multicellular organisms are not confronted with a problem of this sort, since the germ track remains separate from the soma and does not undergo differentiation. A mechanism (perhaps somewhat special for the Protozoa) must have developed in the course of evolution for bringing about the de-differentiation of the cytoplasm at the time of conjugation in Paramecium. However, a line maintains itself in its differentiated state in the course of asexual reproduction. It must therefore contain some sort of cytoplasmic material which can reproduce and multiply in the course of asexual reproduction, and which serves as the physical basis for the inheritance of differentiation. But since there is de-differentiation of the cytoplasm (at least with regard to many traits) with each sexual cycle, it is evident that the self-reproducing material above referred to must disappear with de-differentiation, and that it must form again *de novo* at the time of re-differentiation. Such substances therefore are not genes; they are autocatalytic in that they produce more material like themselves in maintaining differentiation during asexual reproduction—a matter to be considered more fully later. Muller (1929) has pointed out the distinction between genes and ordinary autocatalysts.

It was probably also because of cell size that the ciliate relatives of Paramecium often came to harbor green algae (Zoochlorellae) as symbionts: only a relatively large cell could furnish quarters sufficiently spacious

for intracellular symbionts as large as Zoochlorellae. This fact might account for the widespread occurrence of the green symbionts in the larger Protozoa in general. Green symbionts are found, for example, in *Ameba viridis,* in many of the Heliozoa and Foraminifera, in almost all the Radiolaria (most of which contain the yellow-green alga Zooxanthella), and in many flagellates. They are especially frequent among the ciliates, among which we might note, as familiar examples, *Vorticella viridis* and *P. bursaria,* both of which are very large.

Now in the course of evolution symbionts might very well have come to interact with the genetic system of their hosts. In particular, it has been suggested that kappa (the killer factor) in *P. aurelia* is a symbiont, related to the green symbionts (Zoochlorellae) found in *P. bursaria* (Altenburg, 1946). This suggestion was based originally on the correspondence in number of green symbionts in *P. bursaria* and the number of kappa bodies demonstrated by Preer (1946, 1948*b*) in *P. aurelia* (about 1200 per cell in each case). But more facts have accumulated in support of the suggestion, two of which are of particular importance. One of these was the discovery that the kappa bodies were large enough to be plainly visible under the microscope (Preer, 1948*a*). This at once ruled out the theory that kappa is a plasmagene derived by fractionation from a nuclear gene; all estimates agree in putting the size of the gene beyond the limits of visibility under the microscope. It is true that only Paramecia of genotype *K* can harbor kappa. But this would not necessarily mean that kappa was modified *K*. It might well be that in Paramecia of genotype other than *K* cytoplasmic conditions are not favorable to the continued growth of kappa.

The relatively large size of kappa is consistent with the symbiont theory, since kappa might well be visible if it was derived from a symbiont which itself was visible. It is true that the kappa bodies are not quite as large as the Zoochlorellae of *P. bursaria,* but it is entirely

possible that they might have become reduced in size since the time of their supposed origin from Zoochlorellae, perhaps as the result of the close symbiotic relationship with their host, involving among other things the loss of chlorophyll in this particular case. In fact, chloroplasts become very small after losing their chlorophyll; they might even totally disappear. Moreover, material stained with a nuclear dye would show only the nucleus of the supposed symbionts, not the cell as a whole, and the nucleus might be very small.

A second important fact that came to the support of the symbiont theory was the discovery, again by Preer, that the division rates of the kappa bodies and of Paramecium are somewhat independent of one another (Preer, 1948*a*). At higher temperatures the division rate of Paramecium is speeded up to a greater degree than is that of the kappa bodies. It is therefore possible (by means of temperature) to reduce the numbers of the more slowly reproducing kappa bodies to considerably less than 1200 per Paramecium and even to eliminate them completely in some of the Paramecia. A very striking fact in parallel is that Zoochlorellae can also be removed from *P. bursaria* by a series of rapid fissions (Jennings, 1938).

The relative independence of kappa and Paramecium is shown by two other facts, in addition to their independent division rates: (1) the selective lethal action of high temperatures on kappa, a temperature of 38° C resulting in the death of kappa but not of the host cell, and (2) the transmissibility of kappa to Paramecia previously without kappa by exposing them to broken-up Paramecia that contain kappa. All these facts point to a relative degree of independence that might be expected of an organism distinct in point of origin and physiological workings from its host, but hardly to be expected of a plasmagene or other body that is an integral part of the genetic system of the host proper.

There are equally strong reasons for ruling out the

theory that kappa is a virus or some other pathogenic organism. The relatively large size of the kappa bodies argues against their being a virus, as usually understood. Moreover, kappa is non-pathogenic and thus unlike any known virus. On grounds of non-pathogenicity also, kappa could hardly be rickettsia. A point to be emphasized here is the rather definite upper limit placed on the number of kappa bodies per cell (about 1200). This limitation in numbers is precisely what might be expected; for a symbiont, in contrast to a parasite, multiplies only to the extent that its numbers are in keeping with the welfare of its host.

If kappa is a symbiont, then up to this point we have no evidence for plasmagenes or for cytoplasmic inheritance in the genetic system of Paramecium proper. However, there is a form of cytoplasmic inheritance in Paramecium itself. This is illustrated by the inheritance of mating type in those stocks of Paramecium which contain the "two-type" mating strains. In these stocks, a given ex-conjugant upon division gives rise to two lines both of which might be plus, or both minus, or one plus and the other minus. These possible combinations of mating types occur purely according to chance and without reference to the mating type of the ex-conjugant itself.

Now, when an ex-conjugant gives rise to two lines of the same mating type (both plus or both minus), there obviously is no segregation of genic material. It is therefore not probable that there is any such segregation when the two lines happen to be of opposite mating type; if there were, then one would expect a given ex-conjugant always to give rise to two lines of opposite type. Obviously mating type is not being determined by any such mechanism. But if there is not segregation of nuclear material, it is highly probable that the macronuclei, as well as the micronuclei, are of identical genotype in the two lines derived from a given ex-conjugant, and that both types of nuclei are derived from the fertilization

nucleus by simple mitosis. Moreover, since the mating type of a new line is often the opposite of what it was before conjugation, it follows that the mating type does not persist through conjugation—the cytoplasm becomes de-differentiated with respect to mating type sometime after the onset of conjugation. Hence the determination of mating types in the stocks in question is not dependent on kappa-type bodies of two specific kinds (one for the plus and another for the minus type); for if it were dependent on such kappa-type bodies, then (contrary to fact) mating type would as a rule persist through conjugation. For the same reason, it could not be dependent on any other type of cytoplasmic body that persisted through conjugation as a specific mating type determiner.

If then mating type of the stocks in question is determined neither by the segregation of nuclear bodies (sex chromosomes or genes), nor by cytoplasmic bodies that persist through sexual reproduction and that are specific for plus or for minus mating type, then there is only one conclusion: mating type must be determined in the two-type strains in Paramecium by the local environment in some sense or another (chemical or physical), and we should have here merely another case of a given genotype with different phenotypic expressions comparable somewhat to the case of sex determination in Bonellia. In the case of Paramecium, mating type is ordinarily determined at the metaphase stage in the division of the ex-conjugant into two (the cytoplasm having previously become de-differentiated). At metaphase the ex-conjugant is constricted into two (transversely), so that it now contains two "fields" of cytoplasm, each with its own macronucleus and each the equivalent of a new cell. The local environment within each field might very well cause differentiation of the cytoplasm with respect to mating type. It might seem that this implies a very narrow restriction (within the microscopic limits of the ex-conjugant) of environmental differences. But

it is precisely such localized differences which cause differentiation of indeterminate eggs (and probably of determinate, also) in the case of multicellular organisms. In Paramecium, a slight difference between the two presumptive division products of the ex-conjugant might very well throw the balance in favor of one mating type or the other.

It is true, however, that each mating type as a rule breeds true so long as it reproduces asexually; that is to say, it remains differentiated as a plus or a minus line. Therefore the differentiated state must have some hereditary basis, since it persists from one cell division to the next. But nuclear genes can hardly be at the basis of inheritance in this case. For we saw that the macronuclei of the two sister lines derived from an ex-conjugant are probably identical genetically, having been derived from the same fertilization nucleus by mitosis (without segregation), and so it follows that any difference between the two mating types must reside in the cytoplasm. It must therefore depend on some cytoplasmic material that is characteristic of each type. Moreover, a single Paramecium can give rise, for example, to over a thousand offspring after just ten cell divisions, and unless the material in question had multiplied it would have become correspondingly dilute. This cytoplasmic material must therefore be capable of increase, but it must somehow cause its own increase, since it is the only material that makes the one mating type different from the other (the two types being alike as regards their genes). The material in question is therefore some sort of *autocatalyst*. But we saw that mating type disappears during sexual reproduction and is then formed anew; the autocatalyst in question would most likely follow a similar course. Now genes proper do not go out of existence at the time of sexual reproduction. Neither are they formed *de novo* with each sexual cycle. Hence the autocatalyst is not genic and can hardly be referred to as a "plasmagene." Since it disappears with

each sexual cycle, it must ultimately depend on nuclear genes in conjunction with the specific local environment for its initiation. In fact, the dependence of the autocatalyst on a nuclear gene is known: the two-type mating strains cannot develop in the absence of a certain nuclear gene (+, allelic to mtI for the one-type strains), as shown in the F_2 from crosses between the two-type and the one-type mating strains.

The autocatalyst above referred to is not in any way to be confused with the kappa substance, for kappa does not reappear in a line once it has disappeared, unless new kappa is introduced from some other Paramecium (through delayed conjugation). Just the opposite sort of thing applies to the autocatalyst.

However, in the *B* group of *P. aurelia,* mating type is seemingly dependent on kappa-like bodies insofar as it does actually persist through conjugation in the *B* group. But it is possible that even here some sort of autocatalyst of a more persistent type, yet not genic in nature, is involved. It would be necessary to know in this connection whether the apparently permanent mating types are really permanent. Perhaps they change about occasionally without mutating. If so, then one type would disappear and the other would be formed anew somewhat in the same way as happens more regularly in the case of the two-type mating strains, but unlike what one would expect of genes or kappa-type bodies.

It has been assumed that the antigenic types (*A, B, C, D*) in *P. aurelia* are due to kappa-type bodies (Sonneborn and Le Suer, 1948). It is true that an antigenic type persists through many conjugations and autogamies and that it remains within its own line through conjugation, just as the kappa bodies do. However, it is again possible that here, as with the mating types in the *B* races, an autocatalyst of a more persistent type is involved and that it requires some unusual environmental condition (such as an antibody) to get rid of it, but that it can again be restored under genic influence in combi-

nation with the proper environment. In order to prove that the antigenic types are really due to kappa-type bodies, it would have to be shown that a line can lose a given type of antigen by speeding up its division rate just as in the case of kappa, itself. It would further have to be shown that a line could not regain its antigenic type once it had completely lost it. Moreover, it would be desirable to know whether the antigenic types, upon being stained, are found to contain visible bodies similar in size and number to those described by Preer for the killer race. Similar considerations would apply in connection with the mating types in the *B* races.[1]

Traits that are artificially induced in Paramecium by environmental agents sometimes persist through many sexual cycles. These are the *Dauermodificationen* of Jollos. They eventually disappear under normal conditions, and so are not due to any permanent genic change. They might, however, be due to non-genic autocatalysts, similar in principle to those that control mating type in the two-type strains, but again of a more persistent type. They could hardly be due to the loss of kappa-type bodies (as when killers become sensitives by the loss of kappa), for their return to normal would imply a restoration of the kappa-type bodies, and we saw that kappa cannot be made to reappear in a line once it has been lost (unless new kappa is introduced into the line by delayed conjugation).

It is conceivable that there are kappa-type bodies for other traits in addition to the killer trait, but if such bodies are symbionts one might often expect competition between them, so that eventually only one type would tend to persist in a given line. As a matter of fact, the number of different kinds of kappa-type bodies in a given

[1] Since this article was submitted for publication (Oct. 27, 1948) it has been reported that the antigenic types can, after being lost, be formed *de novo* under genic influence (Sonneborn, 1949), and it is therefore evident that they are due to non-genic autocatalysts, an interpretation which Sonneborn now accepts in effect, though he regards the bodies in question as a kind of gene.

Paramecium does appear to be very limited, since kappa itself (including its mutant forms) is the only well-established body of its kind.

A seemingly strange situation in the genetics of Paramecium is the fact that in the *A* races (those without kappa-type bodies) all known cases of inheritance are Mendelian, but in the *B* races (those with kappa-type bodies) all known cases are cytoplasmic. This has been interpreted as indicating a fundamental difference in the nature of the genic material and in the mechanism of inheritance in the *A* and *B* races. It would indeed be strange if the usual Mendelian mechanism had been displaced in the *B* races by another mechanism (of kappa type), or if it had been relegated to a position of secondary importance and rendered impotent as an hereditary mechanism, particularly since the *B* races are very closely related to the *A* races, in which the Mendelian mechanism still holds good; and further since the *B* races possess a nuclear structure that is visibly identical with the one possessed by the *A* races and that serves in them (the *A* races) as the material basis of Mendelian inheritance.

In this connection it should be noted that there are not many known cases of simple clear-cut Mendelian inheritance even in the *A* races of *P. aurelia*. This is due partly to the fact that many of the differences between the various lines of Paramecium (made pure by autogamy) are of a quantitative nature and are probably due to multiple factors. Crosses between such lines would therefore not give evidence of simple Mendelian inheritance. By contrast, kappa would show clear-cut cytoplasmic inheritance. But actually the vast majority of traits would still be gene-controlled and the usual mechanism of inheritance would still operate.

If the above account is correct, then there is nothing unusual about the genic system of Paramecium proper. The reduction divisions take place as usual for other organisms before gamete formation (during conjuga-

tion, autogamy, or cytogamy), and they are accompanied by Mendelian segregation. There is no conclusive evidence for segregation at any other time. Neither is there conclusive evidence for plasmagenes, if by this term we mean genes within the cytoplasm belonging to the genetic system of Paramecium proper and not to a symbiont.

In conclusion, then, the ciliates conform with other organisms in regard to the fundamental processes of heredity and development. Nevertheless, the findings of Sonneborn and his co-workers rank among the important discoveries in modern genetics, in that they reveal the amazing extent to which symbionts might become enmeshed in the heredity and development of their host, and in that they call attention to experimental findings which can be interpreted as confirmatory evidence for the theory that the inheritance of differentiation in asexual reproduction is due to autocatalysts of a non-genic nature.

Summary

Accumulating evidence supports the theory that kappa is a symbiont, rather than a plasmagene. The inheritance of differentiation in Paramecium is not due either to kappa-type bodies or to plasmagenes, but more likely to autocatalysts of a non-genic nature. This is indicated by the fact that in the two-type mating strains the material basis of differentiation (into a plus or a minus type) is inherited during asexual reproduction but disappears at conjugation, and is then formed *de novo* presumably under the influence of nuclear genes in conjunction with the local environment. It is possible that the same explanation applies to mating type in the *B* races, as well as to the four antigenic types (*A, B, C, D*), but that in these cases the autocatalyst would be of a more persistent type. It would, however, still be non-genic, provided it could arise *de novo* after having disappeared.

It is extremely improbable that a totally new mechanism of heredity, cytoplasmic in nature, has displaced

the Mendelian mechanism in the *B* races, particularly in view of the similarity of their chromosomal behavior to that of the *A* races, in which the usual mechanism of Mendelian inheritance is found.

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