# THE CILIATE MACRONUCLEUS

By B. R. SESHACHAR and K. V. SRINATH

(*University of Mysore, Central College, Bangalore*)

IT is now fairly clear that so far as staining reactions are an indication, the ciliate macronucleus contains a large amount of desoxyribose nucleic acid.[1] This is a matter of major significance, for it has been known for a long time that no chromosomes are formed in it during division, which is of the amitotic type. The occurrence, therefore, of a large quantity of desoxyribose nucleic acid in a nucleus which admittedly does not form chromosomes deserves an explanation; for in other organisms, this has been seen to occur only in association with the chromosomes.

Chromosomes have, however, been reported to be formed in the macronucleus of *Tetrahymena galeii* by Painter[2] and earlier by Hegner and Holmes[3] in *Balantidium*. But these would appear to be exceptions; for normally the division of the ciliate macronucleus is by the direct amitotic type, without chromosome formation. In species of *Epistylis* with which the authors are particularly well acquainted, it is indisputably so.

The occurrence of desoxyribose nucleic acid in the ciliate macronucleus leads one to conclude that it exists there in a condition and relationship very different from those in which it is present in the metazoan nucleus. Recent work[4] on the behaviour of the nucleus in mitosis has shown that the polymerization of the nucleotides is dependent on the protein framework of the chromosome. In fact, the chromosome thread (protein) controls the polymerization of its thymonucleic acid charge, and if the chromosomes are not formed in the ciliate macronucleus, it is more than likely that the reason lies in the extent or nature of its protein content. This in turn is related with the reproduction of the thread, and so the division of the nucleus itself. It is clear, therefore, that the whole chain of events and reactions of mitosis hinge on this central fact of the protein content of the nucleus; and if, in spite of the presence of one of the essential constituents of the chromosome, *i.e.*, desoxyribose nucleic acid, *the chromosomes are not* formed by the macronucleus, it is clear that the other constituent, *i.e.*, protein, is in some way deficient. In fact, one is led to the possibility of visualising the ciliate macronucleus as a body which is either partially or completely lacking in protein, and that that is responsible for the non-formation of the chromosomes by it, and in turn, for its amitotic division.

If it is true that the desoxyribose nucleotides occur in the macronucleus in an unpolymerized condition and unrelated to protein, then this would offer the first instance, unique among animals, where desoxyribose nucleic acid exists outside of and dissociated from the chromosome thread. But it is also significant that almost alone among normal nuclei of animals, the macronucleus of the ciliate is the one that unquestionably divides by amitosis.

The growth and reproduction of the macronucleus offer other interesting points. Whatever the condition in *Tetrahymena*,[2] the macronucleus of most ciliates, and particularly of *Epistylis*, divides at every binary fission, by a simple process of constriction, and each daughter individual has a macronucleus much smaller than that of the parent. This means, a period of growth must follow binary fission during which the macronucleus must enlarge. Growth of the macronucleus must involve the addition of material to it, which we have seen, is largely desoxyribose nucleic acid. It can come from one of two sources. The existing nucleotides in the macronucleus of the daughter individual must reproduce; or new nucleotides, manufactured in the cytoplasm, must find their entry into the nucleus through the nuclear membrane. In the one case it would be a multiplication of desoxyribose nucleotides within the nuclear membrane; in the other, the ribose nucleotides, manufactured in the cytoplasm, must get converted into those of the desoxyribose type on their entry into the nucleus.

If the former possibility is admitted, it will provide the first instance of the multiplication of desoxyribose nucleotides outside the protein medium of the chromosome, where only they are known to do so. This, however, is not surprising, for nucleic acid has been shown[5] to possess the inherent property of self-multiplication.

The second possibility is full of interest. That cytoplasmic nucleotides occur in a variety of animal cells is now fairly well known. That they contribute to the nucleic acid content of the chromosome at mitosis is also clear, and evidences have been presented for a transference of cytoplasmic nucleotides into the nucleus at every mitotic cycle, through the nuclear membrane during early prophase, and more especially at pro-metaphase, when by the breaking down of the nuclear membrane, the cytoplasmic and nuclear materials are in confluence. So it is not surprising that ribose nucleotides pass after every binary fission into the macronucleus, become converted into those of the desoxyribose type and so augment the nucleic acid content of the macronucleus. But it is significant that a situation analogous to pro-metaphase occurs at no time in the history of the ciliate macronucleus, for never does it lose its nuclear membrane, nor does its material ever come in direct and open communication with the cytoplasm. Hence, if cytoplasmic nucleotides pass into the nucleus, they must do so through the nuclear membrane. Painter[2] has recently reported the presence of large quantities of ribose nucleotides in the cytoplasm of ciliates, and the formation of macronuclear buds and their disorganization in the cytoplasm[2,6] must release large quantities of nucleic acid there. These would find their way back, ultimately, into the nucleus at the time of its growth.

There is still a final point to which we would refer, *i.e.*, the origin of amitosis of the

macronucleus. It is to be assumed that in a normal nucleus capable of mitosis, a definite protein-nucleic acid ratio exists, and should be maintained. It is only on the basis of this assumption that the interaction between these two substances makes mitosis possible. Quantitative studies have shown that the chromosome which is more or less entirely a nucleoprotein, contains a 40:60 ratio of nucleic acid and protein.[7] Therefore it would seem necessary that a certain basic protein equipment would be required for the building of a chromosome. Any disturbance of this protein-nucleic acid ratio must have a deterrent effect on mitosis and must produce a condition when mitosis does not occur at all or is so disturbed that it degenerates into amitosis. On this view we may find an answer to the behaviour of the ciliate macronucleus. Of the two substances which alone are known to form the chromosomes, one of them, *i.e.*, desoxyribose nucleic acid, is present in it. And yet no chromosomes are formed. The reason probably is, as set out earlier, the protein-nucleic acid ratio has been upset, with the result that the chromosomes are not formed, and no mitosis occurs.

Why and how this ratio is upset may now be briefly examined. The behaviour of the conjugating ciliate immediately after syngamy appears to provide an answer. The synkaryon is capable of mitosis and divides a number of times. A distribution of the resulting nuclei among the daughter individuals takes place so that each comes to possess the normal nuclear equipment. Concomitant with this is the enlargement of some of the nuclei to become the macronuclei, while the others that do not enlarge, remain as the micronuclei. It is significant that the nuclei which do not enlarge retain the faculty for mitotic division, while those that do, lose it.

We would regard this enlargement as a process of acquisition of cytoplasmic nucleotides. On this basis, it is clear that against a known and fixed protein equipment of the original small nucleus, the addition of enormous quantities of nucleotides has so overloaded it with nucleic acid that the functional ratio between it and the protein has been upset, with the result that the macronucleus has been dispossessed of its vital attributes of chromosome formation and mitosis.

---

1. Seshachar, B. R., and Srinath, K. V., *Nature*, 1946, **158**, No. 4021, 750. 2. Painter, T. S., *Trans. Conn. Acad. Arts and Sci.*, 1945, **36**, 443. 3. Hegner, R. W., and Holmes, F. O., *Amer. Journ. Hyg.*, 1923, **3**, 252. 4. Darlington, C. D., *Nature.*, 1942, **149**, No. 3768, 66. 5. Claude, A., *Biol. Symp.*, 1943, **10**, 111. 6 Seshchar, B. R., *Curr. Sci.*, 1946. **15**, 198. 7. Mirsky, A. E and Pollister, A. W., *Biol. Symp.*, 1943, **10**, 247.