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EVOLUTION OF CELL AND CHROMOSOME IN EUKARYOTA*

A. K. SHARMA

Centre of Advanced Study (Cell and Chromosome Research), Department of Botany, University of Calcutta, Calcutta.

In a discourse on the evolution of an eukaryotic cell and chromosome, it would be necessary to deal with the structure of prokaryotes, their fundamental differences from eukaryotes and finally to discuss the ways through which the eukaryotic system has developed.

Structure of Prokaryota :

In Prokaryota, the gene bearing structure is merely a DNA molecule or genophore. The length of the genophore or DNA molecule reflects the number of genes present and there is very little of non-genic material. Evidently, the linear array of genes arranged in one linkage group might have developed a strong selective advantage.

In absence of a typical mitotic mechanism for separation, such linear aggregation of genes in one molecule facilitates their equitable distribuiton in the two daughter cells arising out of fission. The DNA molecule remains attached with the membrane and replicates. With the elongation of the cell membrane during fission, the sister molecules become separated from one another. This forms an effective mechanism for each sister cell to have a complete copy of the master plate encoding genetic information. In view of the almost complete absence of redundant genes, it is imperative that the cell, for its very survival and growth, needs the entire genetic thread which contains all the vital genes for their life processes to continue.

The variability is generated in the prokaryotic system through transformation, transduction and conjugation in addition to mutation. One of the unique features, however, in gene transfer is that a part of the donor gene can be unidirectionally transferred to the receptor. The recombination opportunities are limited and often need a vector, as in case of transduction. This is in strong contrast to eukaryotic genetic system where recombination affords ample opportunities for generating variability.

Structure of Eukaryota :

In Eukaryota, the chromosome structure is complex with morphologically and functionally differentiated segments of chromosomes. Even in the genome, there are functionally differentiated sets of chromosomes such as nucleolar and non-nucleolar organizing ones, all of which are encased within a nuclear membrane, a structure unknown in the prokaryotic system.

The prokaryotes, as the fossil evidences go, have been traced back to nearly three billion years, whereas, the eukaryotes to one billion years. In spite of the absence

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of any fossil evidences connecting the two, the relationship between these two principal groups have been assumed in view of the universality of the genetic code.

In the eukaryotic system, in addition to nucleus and nuclear membrane, a number of organelles have evolved, all of which do not necessarily have membranes of their own. Lysosomes, peroxisomes and several secretion granules may have a single membrane. Ribosomes do not have a membrane, but the two essential structures—chloroplastids and mitochondria have well defined membranes of their own. Except for Trichomonads, all eukaryotes have a distinct membrane and contain mitochondria, whereas chloroplastids are essential organelles in green cells of plants.

Origin of organelles :

Several views have been expressed regarding the origin of eukaryotic system.

The endosymbiotic theory (Sagan, 1967 ; Margulis, 1970, 1976 ; Reinert and Ursprung, 1971 ; Evstigneev, 1975 ; Buclow, 1976 ; Hanson, 1976 ; *vide* Sharma, 1978) visualizes the origin of complex eukaryotic cell from prokaryotic endosymbionts. It is suggested that ancestral forms included both heterotrophic and photoautotrophic types, the latter having the property of utilizing solar energy. In course of evolution, an amoeboid prokaryotic form engulfed an aerobic respiring bacterium and a mechanism of endosymbiosis developed. Similar engulfing resulted into endosymbiosis with photosynthetic prokaryotes such as blue green algae or photosynthetic bacteria, some of which were even motile. The gradual evolution resulted into the loss of motility of the cells and semiautonomy of mitochondria and the chloroplastids. The relationship permitted the cells to utilize solar energy responsible to carry on photosynthesis as well as other measures of metabolism. Lynn Margulis visualizes in addition, an intermediate symbiotic relationship with *Spirochaete* type of prokaryotes to acquire motility. The evolution of plants was associated with the capture of photosynthetic ability of bacteria, whereas animal evolution did not involve any such mechanism.

Several authors have presented evidences of the endosymbiotic origin of cell organelles, including chloroplastids, mitochondria, microtubules, centriole and flagella. The similarities have been shown in the nature of the genetic material, proteins and protein synthesizing systems (Schnepf, 1971). The DNA of chloroplastids and mitochondria are double stranded and circular like those of bacteria. Their replicating mechanisms, too, are identical. To some extent even blue-green algae have a similar structure (Remsen *et al.*, 1968 ; Bennett and Radcliffe, 1975). The grana and stacking of photosynthetic lamellae of chloroplastids find similarity with bacteria like *Ectothiorhodophora mobilis*. The 70s ribosomes and their subunits of chloroplastids and mitochondria of prokaryotes are quite different from those of 80s eukaryotic cytoplasm. All these evidences suggest an endosymbiotic.

However, such an origin has involved a significant loss of autonomy of the organelles during evolution. It has been shown that the fabric of the mitochondria, its outer envelope and several proteins are synthesized outside the mitochondria under nuclear genetic control (Baxter, 1971). No doubt, DNA of mitochondria can undergo recombination but its transmission is dependent on the nucleus, as best exhibited in *Saccharomyces*, human and man-mouse hybrid systems (Wilkoe,

1973 ; Saccone and Quagliariello, 1975). In fact, the degree of autonomy in the genetic system of mitochondria can be judged from the very fact that in animal systems its protein synthesizing capacity is confined to only 30 genes (Kislev and Eisenstadt, 1972). Similar nuclear control has been noted in species of *Paramoecium* and *Saccharomyces* (Beale and Knowles, 1976 ; Trembath *et al.*, 1975 ; Tzagoloff *et al.*, 1975).

As compared to mitochondria, there is a considerable degree of autonomy of the plastids. In *Euglena*, the protein synthesizing capacity is restricted to 300 genes in chloroplastid DNA. In *Chlamydomonas*, however, several photosynthetic enzymes have been shown to be under nuclear control. Bogorad and others (1975) have suggested a dual control for chloroplastids, two being located in the chloroplastids and one in the nucleus.

Even though there are strong evidences in support of the organelle origin through endosymbiosis, there is an alternative idea of the origin of mitochondria by differentiation from portions of a single ancestral prokaryotic cell (Stanier, 1970 ; *vide* Taylor, 1976). A non-symbiotic origin of chloroplastids and mitochondria has been suggested by Mahler and Raff (1975). Cavalier-Smith (1975) has visualized the origin of eukaryotic system through a single-celled, facultative, non-nitrogen-fixing blue-green alga. All the organelles have been visualized to have originated through cell compartmentation (Fogg *et al.*, 1973 ; Oakley and Dodge, 1974 ; Pickett-Heaps, 1974). Just as the nucleus, the lysosomes, etc. evolved through the cell structure itself, the need for an efficient machinery for respiration ensured by greater volume devoted to respiratory chain in the plasmalemma might have proved to be of high adaptive value.

Moreover, the mitochondria, at least in certain organisms, such as in yeast have been shown to have no DNA, though they consistently produce new mitochondria. In spite of the defective system, such an occurrence is regarded as going against the concept of bacterial origin.

For chloroplastids, the concept of endosymbiotic origin appears to have a strong foundation. Recent researches have even shown that chloroplastids can be reproduced outside the cell in cell free culture, indicating their capacity for independent existence.

The fundamental difference between the theory of endosymbiosis and the simple prokaryotic origin involves a distinction between parallel and convergent evolution. The endosymbiosis theory implies that the prokaryotic organisms involved in symbiosis resulting into mitochondria and chloroplastids still maintain their similarities due to parallel evolutionary modifications. The way through which such a state can be maintained through several billions of years has no precedence. The concept of organelle origin through differentiation within single cell structure implies the origin of the systems through convergent evolution because of similar selection pressures on different gene components, in organelles and prokaryotes. The apparent similarities are due to convergent evolution and merely superficial.

The extent to which similar selection pressure can be operative in differentiated and non-differentiated systems is difficult to visualize. The simple prokaryotic origin of eukaryotic cell presuppose the genes of eukaryotes have evolved from prokaryotes, the evidence being derived mainly from the universality of the genetic code. Recent findings indicate that genes of eukaryotes *vis-a-vis* the chromosomes

consist of contiguous and non-contiguous base sequences unlike prokaryotes. There are series of intervening base sequences of varying length termed *introns* between sequences which are translatable or *exons vide* Darnell (1978). The base sequences from the DNA during gene action are first transcribed in its entirety including introns and exons and from this primary transcript, selected pieces are cleared or spliced off and the translated sequences are further ligated. Due to this "splicing" property—so characterisitc of eukaryotes—the translated transcript is much shorter than the primary transcript. Adenovirus infected tissues show a series of 13 long primary transcripts, rabbit hemoglobin genes possess introns of 550-600 base pairs and tRNA of yeast has an intron of 15 base pairs. Innumerable evidences of this type indicate that the *splicing* off property is universal for all higher organisms.

Along with this outstanding "splicing" property, other characteristics which differentiate an eukaryotic genetic strand from a prokaryotic one involve also the processing of messenger RNA. In eukaryotes, the mRNA is always capped at 5'terminus as well as there is a long polyadenylic acid sequence (Poly A) attached to the 3 terminus. These features appear after the formation of primary transcripts and as such are products of post transcriptional events.

The theory of the origin of "splicing" property goes back in a large measure to the concept of the origin of eukaryota. According to some authors, the splicing property arose simultaneously with due evolution of eukaryotes from prokaryotes, the non-contiguous sequences arising anew, and the insertion of control elements being an associated feature just as transposons of maize. On the other hand, several authors consider that those inter-, vening sequences were present since the beginning of evolution of eukaryota, and both pro and eukaryotes had independent lines of evolution. The proponents of the latter theory discard the concept of inesertion and late origin on the basis that it would require innumerable mutations which is rather unusual for such an universal eukaryote character. The similar type of introns in rabbit and mouse is also an evidence in favour of independent origin as it would have required a long antecedent period of evolution to have such similar structures established in their DNA. Pontecorvo's theory of discrete control elements located in different segments for the expression of one character also goes against a typical polycistronic operon type of functioning in eukaryotes. All these evidences taken in conjunction with the fundamental differences of pro and eukaryotes as discussed above, led Darnell to postulate an independent origin of eukaryota. An argument advanced against the prokaryotic origin is the absence of any rationale of abandoning a simple coordinated genetic structure in a single genetic thread of prokaryotes in favour of a complex inter-chromosomal mechanism on eukaryotes. In eukaryotic system, complexity is further provided by the evidence that chromatin is composed of a complex of histones and DNA organized into nucleosomal subunits. All the sequences, including the transcribable ones, constitute these subunits. The factors which determine, facilitate or restrict transcription are yet to be explored. The special features of transcriptionally active chromatin sequences need to be analysed. The importance of histones even in derepression of gene activity is gradually realized. It is claimed that acetylation, phosphorylation or methylation of basic residues of histones may be responsible for

depression. Thus, in absence of definite evidences of a convergent evolution, the evidences in support of an independent origin of eukaryota, and the fact that mitochondria and the chloroplastids have clear similarities in their genetic complement, the theory of endosymbiosis stands on a stronger foundation with the possibility that one of the partners might not have been prokaryote with its typical genetic complement.

Whatever might be the mode of origin of the cell organelles in a eukaryotic system, their chromosomal control can hardly be questioned at present. The way through which such a control has originated, resulting into semi-autonomous state of the organelles, is yet to be investigated.

Nuclear membranes :

In the origin of chromosomal control of cell organelles, the importance of a nuclear membrane in eukaryota demarcating the genetic material or more precisely chromosomal DNA, from the cytoplasm is of special significance. There are several lower forms of eukaryotes, such as certain fungi, where nuclear membrane is present, but the centrioles, equatorial plate and spindles are absent. The nuclear membrane in eukaryotes serves as an intermediary in the nucleocytoplasmic transfer of gene products as well as in the formation and perpetuation of mitochondria, chloroplastids, endoplasmic reticulum and other organelles (Kaufmann and Gay, 1958 ; *vide* Kasper, 1974 ; Agutter *et al.*, 1976). Evidences indicate a sort of continuous membrane system within an eukaryote cell. Chiarelli (1974) claimed that the chromosomes remain attached to the annuli of nuclear envelope, whereas the importance of the latter in chromosome movement and transportation of metabolites has been suggested by Kubai (1975), Berlin and Oliver (1975) and others. With the gradual evolution of complexity of differentiation, the delimitation of the genetic material by an envelope possibly became imperative. Such a mechanism ensured the replication and transcription of genetic material, unhampered by intra and extracellular factors.

Suggested mode of origin of control :

The mechanism through which the chromosomal control on the cell organelles has evolved is yet not fully explored. Evidences from different organisms indicate that the organelles originating as independent units had to transfer certain genetic units of control to the host chromosome or more precisely, genetic thread in the nucleus during the course of evolution. In spite of absence of any direct evidence of the transfer of controlling units to the chromosome, the analogy can be drawn on the prokaryotic gene transfer to eukaryotic chromosome during malignant transformation. Further, the hybridization of mitochondria and nucleus reported by DuBuy and Riley (1967) is significant. Such hybridization might have involved repeated segments retaining homology between mitochondrial DNA and nuclear DNA. It has been suggested (Sharma 1976, 1978) that in the course of evolution from prokaryotic to eukaryotic system, certain parts of the genome having vital controlling genes of mitochondria and plastids or their precursors were transferred to the chromosome. This transfer was of selective value as it ensured harmony and synchrony of the cell as a whole. The insertion of extra chromosomal genic elements in the chromosomes might have been facilitated due to presence of non-contiguous sequences.

Evolution of complexity of chromosomes :

The chromosome structure of the eukaryotes with its vast complexity has gradually evolved in which Dinoflagellates might have represented an intermediate step. In this group of algae, the nuclear membrane delimiting the nucleus and cytoplasm is present, but the structure and behaviour of chromosomes show primitive features (Godward, 1966 ; Soyer and Haapala, 1974 ; Loeblich, 1976).

The chromosomes are morphologically identical to eukaryotic chromosomes, but chemically different in the absence of histones. In the separation of chromosomes to the two poles in absence of a mitotic mechanism, an intermediate behaviour is noticed. The chromosomes attach themselves to the periphery of the membrane which lengthen with the extension of rigid channels in the cytoplasm controlled by microtubules. The microtubules, however, are not formed within the nucleus.

The presence of several linkage groups or several chromosomes in the eukaryota is directly related to an efficient transport system on mitosis assuring of an equitable distribution of genes in the daughter nuclei. Such discrete linkage groups also add to the efficiency of recombination during sexual reproduction, for which a perfect mechanism has been evolved. In order to have an efficient recombination mechanism, non-contiguous sequences facilitating transfer proved to be of great advantage.

The evolution of complexity of chromosome structure has been associated initially with the delimitation of certain segments meant for generalized function such as spindle organization. In the primitive form, such as Conjugales, as well as species of *Luzula* in Juncaceae, the centromere structure is diffused or polycentric where a c ommon function is performed by a large number of segments. The localization of centromere thus represents an evolutionary advance.

Simultaneously with the progress of specialization of chromosome structure, there has been a development of euchromatic and heterochromatic segments, the latter mainly being concerned with the quantitative genes. Such heterochromatic segments, in genera like *Trillium, Fritillaria* and *Paris*, are present in large numbers. Such a state may be considered as less specialized in view of the fact that a common function is performed by several segments. The localization of heterochromatin in mostly centromeric and telomeric regions, noted in most of the advanced forms is an indication of evolutionary progress. Similar advancement has also been noted with the decrease in the number of nucleolar segments in otherwise diploid species where a single nucleolar organizing region is capable of organizing the nucleolus in the haploid set.

Simultaneously with the gradual advance in the morphology of chromosomes delimiting functional segments, there has been a progressive differentiation of sequence complexity of DNA. Since the discovery of the existence of repeated sequences by Crick in eukaryotic chromosomes, such repeats have been noted in heavy amounts in majority of the organisms. They are present as major, moderate and minor repeats in addition to unique sequences. Such sequences are now regarded to have some function related to control.

Recent studies have yielded discrepant results regarding correlation of the amount of DNA and evolutionary status of an eukaryote species and its degree of differentiation. This is possibly because of the high amount of repeated sequences of DNA in the chromosomes of higher organ-

isms. Such genetic redundancy has been noted in plants, animals and even human systems. Information with regard to such additional genetic elements is still nebulous, but the indications are that their occurrence is univerasl in eukaryotic systems. Their very existence may suggest a strong selective advantage and recent studies indicate their role as spacers, loci of accumulation of mutations or even control of differentiation. Another factor of special importance is the absence of correlation between the chromosome number and genetic complexity and adaptability of the species. Some of the additional genetic elements might have simply contributed to the tolerance of it, without adding to the dosage of genes containing unique qualitative characters. The best example is provided by the family Compositae, which, in majority of the taxa, has a very low chromosome number, though as a taxon, it is ranked as highest among the dicotyledons. Similarly, the haploid set of the human genome contains 3×10^9 base pairs as against lilies and amaryllids which have nearly 20 times DNA, as compared to that of human genome.

Lately, in order to account for the additional amount of DNA, the term 'selfish DNA' has been suggested (*vide* Doolittle and Sapienza, 1980 ; Orgel and Crick, 1980). Such DNAs without any phenotypic effect, may not have any selective advantage. They have developed a strategy for survival such as replication in a congenial cellular environment. No function is to be attributed to it. Evidences have sought for in transposons or transposable elements through which, their maintenance and survival at different loci of chromosomes is ensured. As such, a large number of middle repetitive sequences as well as introns, once established may have a long life expectancy. As evolution is not anticipatory, their potential role in evolution need not be considered as a measure of their function.

However, it is true that evolution is not a premeditated process, but the presence of such a vast amount of molecular sequences surviving against the rigors of selection, be it phenotypic or cellular, can hardly be visualized. As the functions of a significant proportion of repeats has already been demonstrated the functions of the rest of the sequences may await a further understanding of the complexity of evolutionary mechanism.

Thus, in a sense, the evolution of chromosome structure has involved the delimitation of functional segments of chromosomes and the emergence of sequence complexity of DNA. The progressive specialization of qualitative complexity of chromosome structure in eukaryotic system is directly correlated with the differentiation of cells, tissues and organs and the metabolic pathways which the genes control.

REFERENCES

AGUTTER, P. S., H. J. MCARDLE AND B. MCCALDIN. 1976. Evidence for involvement of nuclear envelope nucleoside triphosphate in nucleocytoplasmic translocations of ribonucleoprotein. *Nature* **263** : 165-166.

BOXTER, R. 1971. Origin and continuity of mitochondria. *Origin and Continuity of Cell Organelles*. Eds. J. Reinert and H. Ursprung. (Berlin : Springer-Verlag) Vol. **2** : 46-84.

BEAXE, J. H. AND J. K. C. KNOWLES. 1976. Interspecific transfer of mitochondria to *Paramoecium aurelia*. *Mol. Gen. Genet.* **143** : 197-201.

BENNETT, J. AND C. RADCLIFFE. 1975. Plastid DNA replication and plastid division in the garden pea. *FEBS Lett.* **56** : 222-225.

BERLIN, R. B. AND J. M. OLIVER. 1975. Membrane transport of purine and pyrimidine bases and nucleosides in animal cells. *Int. Rev. Cytol.* **42** : 287- 336.

BUCLOW, D. E. 1976. Phylogenetic origin of the chloroplast. *J. Protozool.* **23** : 41-47.

CAVALIER-SMITH, T. 1975. The origin of nuclei and of eukaryotic cells. *Nature.* **256** : 463-468.

CHIARELLI B. 1974. Bands or chromomeres : A functional interpretation of banding and speculation about a model of nucleolus and chromosome organization in the eykaryotes. *Leiden Chr. Conf.* 14.

DARNELL, J. E. 1978. Implications of RNA. RNA splicing in evolution of eukaryotic cells. *Science.* **202**, 257-300.

DUBUY, H. G. AND F. L. RILEY. 1967. Hybridization between nuclear kinetoplast DNAs of *Leishmania emrieti* and between nuclear and mitochondrial DNAs of mouse liver. *Proc. Nat. Acad. Sci.* (*Wash.*) **57** : 790-797.

DOOLITTLE, W. F. AND C. SAPIENZA. 1980. Selfish genes, the phenotypic paradigm and genome evolution. *Nature.* **284** : 601-603.

EVSTIGNEEV, V. B. 1975. On the evolution of photosynthetic pigments. *Origins of Life.* **6** : 435-439.

FOGG, G. E., W. D. P. STEWART, P. FAY AND A. E. WALSBY. 1973. *The blue green algae.* New York : Academic Press.

GODWARD, M. B. E. Ed. 1966. *The chromosomes of the Algae.* Edward Arnold, London.

HANSON, E. D. 1976. Major evolutionary trends in the animal protists. *J. protozool.* **23** : 4-12.

KASPER, C. B. 1974. Chemical and biochemical properties of the cell nucleus. In : *The Cell Nucleus.* Ed. H. Busch. Academic Press, New York, Vol. I, pp. 349-384.

KAUFMANN, B. P. AND H. GAY. 1958. Nuclear membrane as an intermediary in gene-controlled reactions. *Nucleus.* **1** : 57-74.

KISLEV, N. AND J. M. EISENSTADT. 1972. *Eur. J. Biochem.* **31** : 226-229.

KUBAI, D. M. 1975. The evolution of the mitotic spindle. *Int. Rev. Cytol.* **43** : 167-227.

LOEBLICH, A. R. 1976. Dinoflagellate evolution —speculation and evidence. *J. Protozool.* **23** : 13-28.

MAHLER, H. R. AND R. A. RAFF. 1975. The evolutionary origin of the mitochondrion—a non-symbiotic model. *Int. Rev. Cytol.* **43** : 2-124.

MARGULIS, L. 1970. *Origin of eukaryotic cells.* Yale Univ. Press.

MARGULIS, L. 1976. The theme (mitotic cell division) and the variations (protists) ; implications for higher taxa. *Taxon.* **25** : 340-391.

OAKLEY, B. R. AND J. D. DODGE. 1974. *J. Cell Biology.* **63** : 322-325.

ORGEL, L. E. AND F. H. S. CRICK. 1980. Selfish DNA—the ultimate parasite. *Nature,* **284,** 604-607.

PICKETT-HEAPS, J. D. 1974. *Biosystems.* **6** : 37-48.

REINERT, J. AND H. URSPRUNG. (Eds.) 1971. *Origin and Continuity of Cell Organelles.* Springer-Verlag, Berlin.

REMSEN, C. C., S. W. WATSON, J. B. WATERBURY AND H. G. TRUPER. 1968. Fine structure of *Ectothior hodospora mobiles* Pelsh. *J. Bacteriol.* **19** : 2374-2398.

SAGGONE ,C. AND E. QUAGLIARICELLO. 1975. Biochemical studies of mitochondrial transcription and translation. *Int. Rev. Cytol.* **43** : 125-165.

SAGAN, L. 1967. On the origin of mitosing cells. *J. Theor. Biol.* **14** : 225-227.

SCHNEPF, E. AND R. M. BROWN, JR. 1971. On relationships between endosymbiosis and the origin of plastids and mitochondria. In : *Origin and continuity of cell organelles.* Eds. J. Reinert and H. Ursprung. Springer-Verlag, Berlin, Vol. 2. pp. 299-322.

SHARMA, A. K. 1976. A new look at chromosome and its evolution. *Proc. Indian Nat. Sci. Acad.* (Silver Jubilee Medal Mem. Lecture). **B42:** 12-24.

SHARMA, A. K. 1978. Change in chromosome concept. *Proc. Indian Acad. Sci.* **87B** : 161-190.

SOYER, M. O. AND O. K. HAAPALA. 1974. Division and function of dinoflagellate chromosomes. *J. microscopia.* **19** : 137-146.

STANIER, R. Y. 1970. *Symp. Soc. Gen. Microbiol.* **20** : 1-38.

TAYLOR, F. J. R. 1976. Autogenous theory of the origin of eukaryotes. *Taxon.* **25** : 377-390.

TREMBATH, M. K., B. C. MONN, G. M. KELLERMAN AND A. W. LINNARE. 1975. Biogenesis of mitochondria. *Mol. Gen. Genet.* **140** : 333-337.

TZAGOLOFF, A., A. AKAI AND R. B. NEEDLEMAN. 1975. Assembly of the mitochondrial membrane systems. *J. Biol. Chem.* **250** : 8228-8235.

WILKIE, D. 1973. Cytoplasmic genetic systems in eukaryotic cells. *Br. Med. Bull.* **29** : 263-268.