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A NEW LOOK AT CHROMOSOME AND ITS EVOLUTION

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Dr. Pal, Distinguished Fellows of the Academy, Ladies and Gentlemen!

May I, at the onset, express my deep gratitude to the Indian National Science Academy for selecting me for the award of Silver Jubilee Medal for the year 1976. I feel greatly honoured and do not consider myself as a worthy recipient. In any case, as an address is to accompany the award, my choice naturally fell on "*Chromosomes*" with which I got acquainted more than three decades back. Our own researches, coupled with the data obtained from other centres, have led to the concept of "Chromosome dynamism"—the basis of supreme control of chromosomes in reproduction and differentiation (Sharma 1975*a*) modifying the basic tenet of constancy in each and every cell of an organism. In the present talk, further data gathered during the last one year*, have been incorporated providing an additional basis for a new look on chromosomes and their evolution.

ORIGIN OF EUKARYOTIC CELL

The chromosome is preferably called the genophore (Ris 1971) in lower organism or prokaryota, where the structure is merely a DNA molecule. The prokaryotic cells differ fundamentally from the higher organisms—the eukaryota, in the absence of nuclear membrane, chromosomal histone, mitotic mechanism for separation, in the presence of a single replicon and in the mode of genetic regulation, leaving aside mitochondria, golgi bodies and such other cell organelles responsible for cell differentiation. The way through which the eukaryotic cells have evolved, is much debated, since fossil evidences of the intermediaries have rarely been found. Even in the absence of such evidences, the relationship between these two major forms of life can hardly be questioned as the genetic code controlling all aspects of heredity has been shown to be universal.

The present day prokaryotes have a long antecedent period of evolution—nearly three billion years (Sagan 1967), through which they have maintained the primitive cell type even after evolution of eukaryotic system. It is quite likely that mitochondria, chloroplastids, basal bodies and other cell organelles have originated from free-living prokaryotes. The larger amoeboid or flagellated forms of cell were involved initially in parasitism, finally evolving an endosymbiotic relationship (Smith *et al.* 1969; Clowes 1971). It is visualized that blue green algae and anaerobic

*A part of the work was carried out during the author's tenure as Jawaharlal Nehru Fellow.

bacteria might have been injected into amoeboid protoplasts. Such a relationship allowed the cells to utilise solar energy and to carry on photosynthesis along with other metabolic systems for securing nutrition.

The development of multicellular forms in the plant system could be visualized from the unicellular independent alga like *Chlamydomonus* having the full capacity for performing all functions (vide Kimball 1970). Similar behaviour is noted in other flagellated green alga like *Eudorina* which forms a colony but is itself quite independent. In a *Volvox* colony on the other hand, there are reproductive and non-reproductive cells and the latter do not have the capacity of forming colonies. Thus another stop towards multicellular organization with initiation of differentiation was achieved.

The importance of membrane system in bacteria is at present poorly understood (Ryter 1968; Knempel 1970), but the association of bacterial genophore possibly at the terminal and initiation points of replication with cell membrane has been noted (Ganesan and Lederberg 1965). The origin of the nucleus, having nuclear membrane with clear delimitation between areas separating the master templates of DNA from the other metabolic zone susceptible to stress and strain of environment was achieved in different stages. An intermediate form might have been represented in Cyanophyceae. This group is not much advanced than bacteria but the differentiated central area contains several replicons replacing a single DNA molecule and is quite separate from the area with protractile granules.

Even several lower forms of eukaryota do not have typical mitosis, shown by the absence of centrioles, equatorial plate and spindle and the distribution of chromatin throughout as in certain fungi. They naturally do not represent degeneracies but rather an offshoot from the main line where sexuality in the true sense did not develop (Dowben 1971).

In the eukaryotic system, the nuclear membrane not only serves the function of delimitation but also has an important role in the gene-controlled reactions, in the perpetuation of mitochondria, chloroplastids, endoplasmic reticulum and other organelles as clearly elucidated in several organisms (Kaufmann and Gay 1958; Bell and Muhlethaler 1964). It is true that chloroplastids and mitochondria represent independent replicating systems in the cytoplasm and their DNA might have been originally evolved from prokaryotic symbionts, as specially evidenced in the nature of DNA molecule and proteins (Edelman *et al.* 1967). It is also true that mitochondrial 70s ribosomes are different from cytoplasmic ribosome-80 types (Stutz and Noll 1967; Hoober and Blobel 1969; vide Schnepf and Brown 1971). But even then their incapability for continued survival without the nucleus as well as the importance of nuclear gene in their protein synthesis indicates their nuclear control, though perhaps, not absolute. For the chloroplastids, three genes have been supposed to be responsible, two being located in the nucleus, and one in the chloroplastid itself (Bogorad *et al.* 1975). Such a control is quite rational in view of their synchronous behaviour during cell division, where chromosomal separation is also associated with the division of cytoplasmic organelles.

Notwithstanding the fact that they originated as independent units, it is hardly suggested that transfer of DNA from precursors of mitochondria and chloroplastids

to nucleus might have occurred during the course of evolution. This suggestion is based on two important evidences:

(*i*) There are ample records that prokaryotic genes can be incorporated into the chromosome of eukaryotic system, the best example being provided by the integration of viral DNA in the chromosomes of mammalian transforming cell lines (Blangy *et al.* 1974).

(*ii*) Occasional hybridization between nuclear and mitochondrial DNA as reported by du Buy and Riley (1967) may be interpreted as due to the remnants of some similar segments (repeats) left out in mitochondria after transfer.

Such a process led to the transfer of some of the controlling genes of the mitochondria and chloroplastids to the chromosomes which ultimately stood the test of selection. The advantage of such a selection could be clearly visualized since it led to harmonious development of the cell as a whole, with the nuclear and cytoplasmic factors being synchronised, the synchrony being controlled at the nuclear level. Other cell organelles too might have evolved a similar system.

Evolution of Structural Differentiation of Chromosomes

Complexity in chromosome structure of eukaryotes gradually evolved through different steps from a prokaryotic system. Even though the intermediaries are rare, the chromosomes of Dinophyceae may be regarded as representing an intermediate step in evolution. Here in addition to the presence of a delimited nuclear membrane, the chromosome structure is well defined excepting that it lacks the histone characterising chromosomes of eukaryota. No mitotic mechanism could be detected. In that respect, it rightly occupies the position of *mesokaryota* in between prokaryotes and eukaryotes (Soyer and Haapala 1974). From the standpoint of chromosome structure, it has yet to attain the complexity of eukaryotic chromosome. At the cytological level, gradually with the onset of mitotic mechanism, the need for spindle attachment region along with several other functions, was felt and certain genes had to be set apart for performing specialized functions, of which centromere organization was one. At the initial stage, such as in *Spirogyra* and other Conjugales, the function was yet to be localized to a specific zone and as such the genes were more or less diffuse. The spindle fibres were organized along the entire chromosome length. Similar behaviour was true for nucleolar organization as well (vide Godward 1965). Such a state of centromere, diffuse or polycentric, is maintained even in some of the angiosperms, such as *Luzula* (Camara 1951) and species of Cyperaceae. The organization of centromere localized at a particular segment as in most plant, animal and human systems represents an evolutionary advance. This was a step towards acquiring efficiency in spindle attachment and chromosome movement, in which a large number of genes performing similar functions were replaced by a single or a group of genes in a specialized segment.

Similar to centromere, evidences of such localization of function through differentiation of chromosome segments could be observed in heterochromatic regions as well. Heterochromatic segments as is well known are of similar nature in different loci and mostly have a common function. The existence of similar controlling

elements interspersed throughout the chromosome length, could be noted in genera like *Trillium*, *Fritillaria* and *Paris*, in the so-called nucleic acid starved areas of Darlington and La Cour (1940). In these genera, the function of heterochromatin is attributed to several genes located in different segments of chromosomes, all performing a common function. But an efficient system, possibly suggesting specialization and advance, is represented in *Vicia faba* where heterochromatic genes are mostly located in one locus of chromosomes. In the evolution of chromosome structure, it need not be assumed that the species as a whole represents a primitive or an advanced condition but rather it indicates a primitiveness or advance in respect of certain functional segments of chromosomes, irrespective of the state represented at other loci. Such localization of functional genes could be recorded in secondary constriction or nucleolar organizing regions as well. The case of *Spirogyra* has already been mentioned where the nucleolar organizers are dispersed throughout the chromosome. Of the angiospermous genera, in species of *Musa* (Chakravarty 1951), *Scilla*, and others, the chromosomes contain a large number of secondary constrictions and consequently a large number of nucleolar organizers. In these cases, the number of nucleoli clearly corresponds with the number of secondary constrictions. As nucleolar organization is a common function of all these segments, delimitation of a single zone for the performance of this function is yet to be achieved. In majority of the species, on the other hand, nucleolar organization has been restricted to a particular segment of one or a few chromosomes, probably representing a state of advancement. Evidence of functional efficiency of nucleolar organization in one segment instead of at several loci has been obtained in the analysis of satellite DNA in higher organisms which would be discussed later.

Complexity in Chemical Make-up of Chromosomes and Role of Different Constituents

A eukaryotic chromosome can hardly be compared with the prokaryotic genophore. In the human system, DNA forms a stiff fibre about 20 Å in diameter and fragments of 100–200 nm can be isolated (Ris 1971). A human haploid cell contains about 1 metre long of chromosome matter, an average chromosome length being 40,000 nm, i.e. 40 times more than the *Escherichia coli* genome. The complexity and interrelationships of different chemical components in chromosome structure have not yet been fully resolved. Such a complexity is fully comprehensible in view of the contradictory obligations that a chromosome is to perform. They are to satisfy the diverse requirements of the cell, arrange for their sequential and phasic growth in harmony, allow continuity, but at the same time leaving enough scope for incorporating discontinuity through mutations. To meet the needs on the one hand of meristematic activity, and on the other, differentiation, where cell division may upset the controlled growth, the behaviour of chromosome is to be differential. It is to undergo equational division in certain organs and reductional in others. It is to allow recombination to secure new adaptations, and check recombination in others, for the maintenance of stability. Mutation is to be accelerated in some and suppressed in others. In its own framework, specific functions are to be allotted to certain genes; other vital activities such as replication of the same genes, on the contrary, are controlled by other genes. Thus an excellent example of

interdependence is manifested in the chromosomes—the most complex organic molecule that one can conceive of.

In order to perform such diverse functions, the evidences indicate that the chromosomes manifest diversity in their chemical make-up and dynamism in their behaviour (Sharma 1973, 1975*a*). This concept of dynamism provides a reasonable basis of chromosomal control of regulation, differentiation and reproduction in eukaryotic system.

Attempts have been made by several authors to draw a clear line of demarcation between chemical components of chromosomes which are responsible for their structural integrity, from those which are ephemeral in appearance connected only with certain aspects of metabolism (Prescott 1970). In addition to DNA, the genetic material, the roles of RNA, histones and non-histones, as well as divalent cations have been discussed in this context.

In the mammalian system, metaphase chromosomes have been isolated and DNA, RNA as well as proteins have been analysed in different species. It has been claimed that protein helps in condensation, control of DNA and RNA synthesis as well as chromosomal integrity (Izawa *et al.* 1963; Prescott 1970). Regarding histone, even though it is mostly associated with the condensed state, its occurrence in dispersed condition in polytene chromosome has also been recorded (Swift 1965, Gorovsky and Woodward 1965). It has been suggested that activation of transcription may involve loosening of DNA-histone link rather than complete removal of histone (MacInnes and Uretz 1967). There is evidence that histone DNA complex is present in super helix which is about 120Å in diameter. Most of the period, DNA is associated with histone (Pardon *et al.* 1967; Davies 1968), and cross-linking of adjacent loops of DNA by histone has been claimed. Interband regions of polytene chromosomes of *Drosophila* have a very low protein content which is claimed to be histone (Swift 1962). Non-histone chromosomal proteins also have been suggested to play a very significant role in regulation of transcription in eukaryota (Stein *et al.* 1974), including even the binding of histone to DNA. The absence of requisite diversity needed in histones to control regulation of a variety of characters has been pointed out. The importance of non-histone protein is realized. but the exact mode of interaction is yet to be established.

The role of proteins in lateral and serial linking of DNA molecules thus helping in the maintenance of chromosome integrity has been suggested. But evidences through autoradiography and digestion through enzymes do not provide any clue to the presence of serial linkers (Prescott 1970). If polynemic models of eukaryotic chromosomes are proved viable, proteins may serve as crosslinkers. Prescott has however argued that the same purpose can even be achieved through interaction of mere DNA molecules.

There is very little evidence that RNA does enter into primary structure of the chromosome (Prescott 1970). Most of the evidences indicate that RNA is associated with the surface of the interphase chromosome as in HeLa cells, human lymphocytes and fibroblasts (Comings 1966) and does not enter into the chromosome skeleton. Earlier (Mirsky and Ris 1947; Sharma and Roy 1956; Sharma and Sharma 1958), RNA was recorded to be an essential constituent of "residual chromosome" responsible for chromosome integrity.

Evolution of Sequence Complexity of DNA

Simultaneously with the increasing complexity of chromosome structure in eukaryotic system as exhibited in the presence of RNA and different types of protein throughout or in certain stages of the divisional cycle, there has been an increasing complexity of the DNA skeleton as well, manifested in the increasing diversification of repeated, highly repeated and non-repeated unique sequences. The repetitive sequences are very common in eukaryotic system, starting from the flagellate to mammals including human cells (Britten and Davidson 1971; Rae 1972; Jones1974). Normally 10–30 % of DNA has been found to be repetitive but a value as high as even 80 % has been noted in *Amphiuma*. Highly homogeneous repetitive sequences located in the satellite DNAs are present in several organisms and their function in relation to synthesis of 20s and 18s ribosomal RNAs has been established (Wallace and Birnsteil 1966). Human DNA has three single fractions of satellites (Jones 1973) which are not thought to be transcribed *in vivo* (Walker *et al.* 1969). Molecular hybridization technique has clearly indicated the role of satellite DNA in the synthesis of ribosomal RNA (Pardue and Gall 1970) and as such nucleolar metabolism. The repeated sequences are also quite common in plants as worked out by different authors (Schwartz and Taylor 1974). Even 70–75 % of chromosomal DNA of rye has been found to be of repeated sequences (Ranjekar *et al.* 1974).

Repeated DNA, if localised in heterochromatic segments as the evidences indicate, may have a significant bearing on the property of heterochromatin. Yunis and Yasmineh (1972) in their model of mammalian constituent heterochromatin has suggested that in evolution of satellite DNA, functionally degenerate ones are lost or maintained in heterochromatin or spread in the euchromatic segments. Constitutive heterochromatin is the region where the new satellite DNAs are emerging. Any DNA sequences not interfering with genetic function, such as transcription, may be set aside in proximal heterochromatin where no sophisticated sequences are necessary. Repetitive sequences may serve as spacers for vital regions (Britten and Kohne 1968). Their presence in centromere and secondary constrictions is of advantage in forming interchromosomal connections. In the eukaryotic system, Dupraw (1970) has visualized suprachromosomal organization in which the chromosomes are interconnected throughout the divisional cycle. Leaving aside the absolute feasibility of Dupraw's contention, the existence of an interchromosomal control in the manifestation of characters in eukaryotes can hardly be denied. The appearance of a character depends on the interaction of large number of genes localised in different loci and may be in different chromosomes which are to function simultaneously.

Comings and Okada (1972) have suggested that if two chromosomes have single copies of synapsing genes with lateral extension of chromatin loops of a synaptonemal complex, the possibility of mispairing is the least. If moderately repetitious DNA (Britten and Kohne 1968) are interspersed throughout rather than in one locus as in satellite DNA, the genes at non-homologous sites may pair posing a serious threat to homologous pairing at the molecular level.

In the evolution of chromosome structure, repeated sequences and satellite DNAs have played a very significant role. In addition to the specialized function

of satellite DNAs containing rDNA cistrons, the importance of repeated sequences as spacers has certainly added to the efficiency of gene functioning. Their existence in such high amount in higher organisms is also suggestive of their role in other mechanisms of control. The arrangement of satellite DNA at one locus and other less repeated sequences interspersed throughout the chromosome without in any way hampering crossing-over, is likely to serve as a model for highly evolved chromosome structure. The presence of such repeats not only confers some amount of flexibility for adaptation but may also serve as loci for accumulation of mutations. At the chromosomal level, repeated sequences are visualized through analysis of banding pattern (Hecht *et al.* 1974; Sharma 1975*b*). Such investigations if correlated with adaptive features may yield significant clues in this direction.

Thus, the complexity of chromosome structure at the eukaryotic level includes sequence complexity of DNA in addition to different types of protein and RNA. Without entering into the debate of transitional and permanent components of chromosomes, it is desirable to view the eukaryotic chromosome structure as a whole, as responsible not only for continuity and reproduction but also for the expression of differentiation of characters. In that case, all these components are to be regarded as playing a vital role in the composition of chromosome even though some of them may not be as stable and permanent as the deoxyribonucleic acid molecule of genes.

Dynamism in Chemical Nature in Relation to Organ Differentiation

This complexity of chemical make-up in the chromosomes of eukaryotes has been associated with dynamism in structure and behaviour during different phases of growth and development. This concept of dynamism has been postulated (Sharma 1975*a*) in view of the numerous evidences gathered from different centres including our own.

Of the components that enter into composition of chromosomes, in several organisms, lysine rich histones are replaced by arginine rich histones and by protamines later, during spermiogenesis (Felix *et al.* 1956; Bloch and Hew 1960). There are also cases, where the substitution may not go up to the protamine level (Das *et al.* 1964). It is also demonstrated that quite a sizeable proportion of sperm protein is non-basic and that the view that DNA packing in sperm is merely due to conversion to polypeptides has been criticized as an oversimplification (Prescott 1970). Ruderman *et al.* (1974) noted that the kind and amount of histone that associates with DNA of chromatin during embryogenesis, differs characteristically from stage to stage. On the basis of orcein banding pattern as well (Sharma 1975*b*) based on DNA protein linkage, similar variability has been recorded.

At the DNA level too, the variability is marked in relation to metabolic DNA. In *Vicia faba, Pisum sativum* and *Nigella sativa*, chromosomes of different organs show clearly different DNA values following Feulgen cytophotometry (Table I) as carried out during the present investigation.

The measurements were carried out at 546 nm under identical conditions of fixing, processing and scoring of data in Reichert Zetopan with microphotometer. The values as may be noted from the table differ significantly in root and shoot tissues. Similar variability is present in other species of plants as well (Sharma,

TABLE I

Values of DNA *in different organs of plants*

Name of species	Organ	Mean Absorbance (%)	Mean Extinction
Vicia faba	Shoot	21.5	0.107
	Root	30.3	0.158
Pisum sativum	Shoot	6.5	0.03
	Root	13.0	0.06
Nigella sativa	Shoot	18.0	0.09
	Root	22.5	0.115

unpublished). Innocenti *et al.* (1975) observed a change in histone/DNA ratio during endoreduplication in metaxylem cell line in *Allium cepa.* In *Petunia hybrida,* metabolic DNA synthesis has been recorded in the beginning of G_1 phase (Essad *et al.* 1975). In sea urchin embryo, there is a 2000 fold increase in nuclear DNA from fertilization to pluteus stage (Scarano 1973). Becak and Becak (1973) have recorded variation in number of secondary constrictions of chromosomes in different stages of development and explained it on the basis of the varying necessity of rDNA cistrons for nucleolus organization in different phases.

All these evidences clearly indicate that eukaryotic chromosomes, maintaining their genetic skeleton, change their pattern during development and differentiation for regulation of all vital activities. This concept of dynamism implies the futility of proposing a universal model of chromosome structure, if the chromosome is to be viewed *as a whole* in eukaryotic system without distinguishing the permanent and non-permanent entities. There is no reason to suppose that chromosomes which are packaged from transmission through germinal line are identical in all respects with the chromosomes of the body cells. Complexity of the mechanism of regulation of differentiation demands a dynamism in structural pattern which the chromosomes fulfil in all respects. Detailed studies are in progress involving cytophotometric analysis of chemical make-up of chromosomes at the *in situ* level of different organs which are providing more evidences of dynamism in chromosome structure.

DYNAMISM IN CHROMOSOME BEHAVIOUR IN RELATION TO DIFFERENTIATION

In the regulation and control of differentiation, dynamism in chromosome behaviour as well, is equally manifested. After the cessation of meristematic growth, differentiation of organs is initiated in the mature tissue, when gene action is supposed to reach the optimum level. It has been recorded from our laboratory (Sen 1974) through inducing division in nuclei by 2, 4, dichlorophenoxyacetate and indolyl acetic acid that such adult nuclei in differentiated region normally lie in both

diploid and polytenic states. Polyteny however was already reported by Geitler (1946) and later confirmed by others as well (Sharma and Mookerjea 1954). In such tissue, gene action depending on transcription being the primary objective, nuclei may lie in a diploid state without undergoing replication, as only one strand of DNA is needed for transcription. But occurrence of polyteny as well, i.e., endomitotic replication of chromosome without cell division, is an index that replication of chromosome becomes necessary when the transcribing capacity of a single strand of DNA is fully exhausted. Such a method of replication meets the need of fresh strand of DNA required for transcription, without at the same time undergoing nuclear and cell division. This unorthodox chromosome behaviour is resorted to in a differentiating system, so that the need for differentiation can be met without increase in the number of cells—an absolute necessity for maintaining a symmetrical growth. This is an excellent example of chromosome dynamism, where the entire behavioural pattern of chromosomes is altered to exert genetic control on organ differentiation.

Dynamism in Chromosome Behaviour in Relation to Reproduction

A change in chromosome behaviour is also marked in species where reproduction is principally or obligatorily vegetative. In such cases, mostly represented in lilies, amaryllids, aroids and several monocotyledonous families, the chances of new combinations and variability become limited due to the absence of a regular sexual method of recombination. Taking the clue from original observations in *Caladium bicolor* (Sharma and Das 1954), it was confirmed from a series of publications from this laboratory (Sharma 1974), that in such asexually reproducing species, the somatic tissue represents a mosaic of chromosome complements in which the normal complement occurs in the maximum frequency (Sharma and Sharma 1959). This inconstancy in the chromosome complement plays a significant role in the origin of new genotypes through their participation in the formation of new daughter shoots as embodied in a new concept of speciation (Sharma 1956). The regular occurrence of variability arises out of non-disjunction and other chromosome behaviour, evidently through genotypic control. This mechanism provides the species with an effective means of securing variability and new genotypes without undergoing the complicated process of sexual reproduction and fertilization. It is an outstanding example of dynamic nature of chromosome behaviour, in which the pattern is altered as soon as the species loses the capacity for sexual reproduction.

A problem which was originally posed while this theory was propounded, involves the nucleocytoplasmic balance which is normally maintained in individual cells. In these cases, evidently such a balance is upset and it was presumed (Sharma 1956) that possibly the balance is maintained in the tissue taken as a whole, rather than between individual cells.

Lately, microspectrophotometric analysis has been carried out here of the DNA content of chromosomes of somatic cells of a number of species (Table II) where such variability in chromosome complement was earlier recorded (Sharma and Bhattacharyya 1954). The data were scored on different individuals of the same species. Identical zone of somatic (root) tissue was taken, stained in

TABLE II

Values of DNA in the somatic tissue in different individuals of Crinum asiaticum

No. of plants	Range of absorbance (%)	Range of extinction values	Mean absorbance	Total absorbance
I	42–24	0.24 – 0.12	31.71	445
II	42–21	0.24 – 0.105	31.60	443
III	41–27	0.235 – 0.135	32.22	451
IV	42–24	0.24 – 0.12	32.28	452

Feulgen solution and measurements were taken at 546 nm using a Reichert Zetopan with microphotometer. It may be noted from the table that in spite of this variability in chromosome number, the DNA content of tissue taken as a whole as measured from a fixed number of random metaphases and nuclei from the tissue, does not show significant variation in different individuals. This confirms the assumption that in these species, the nucleocytoplasmic balance is maintained in the tissue rather than in individual cells. Confirmation of this behaviour is also being sought through isolation technique. The dynamic property of chromosome has set out a pattern in which in spite of the variability needed for evolution of new forms, the nucleic acid balance is not upset. Other authors have also recorded that though heteroploid cells are characterized by variability in chromosome number, cytogenetic, cytophotometric and flow microfluorometric data show an overall constancy in DNA amount and the mammalian system has been visualized to exist in states other than the traditional entity state (Kraemer *et al.* 1972).

CONCLUSIONS

These evidences indicate that we have come a long way from chromosome theory of heredity, substituting and modifying it further and further through varied lines of approach. The data have no doubt strengthened the concept of supreme control of chromosomes on all aspects of metabolism. But in order to exert this control, the change in structural pattern of chromosome, both physical and chemical, maintaining the stability of the genetic material, became inevitable. Simultaneously, there has been a shift in behaviour to meet demands of growth, development and reproduction which are sequential and phasic in eukaryotes. This evolution of complexity and flexibility as embodied in this new concept of dynamism has been the principal feature of evolution from lower to higher forms of life. A critical analysis of chemical and physical nature at the ultrastructural level, as well as behaviour and pattern at different stages, is revealing more evidences of this dynamic behaviour. An understanding of the mechanics of dynamic control is essential for a solution of the longstanding problem of regulatory systems in higher forms of life.

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