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REVIEW ARTICLE

Post-cellular biotic evolution in a nutshell

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Abstract
The minimum requirements for post-cellular evolution from prokaryotes towards multicellular eukaryotes are outlined. The main steps are: formation of a true nucleus harboring linear chromosomes with centromeres and telomeres, establishing of mitosis and of sex via meiosis and fertilization, endosymbiotic gain of mitochondria and plastids, and epigenetic differentiation of non-separated cells into tissues of multicellular organisms. Erroneous DNA double-strand break repair and cell fusion are postulated as main drivers of eukaryotic evolution. Advantages versus disadvantages of eukaryotes compared to prokaryotes are discussed.

Keywords Eukaryotes · Endosymbiosis · Evolution · Mitosis · Meiosis · Linear chromosomes

Introduction

This parsimonious sketch is dedicated to the memory of the outstanding Indian botanist and cytogeneticist Arum Kumar Sharma at the occasion of the centenary of his birthday in 1924. He was a hero of science, the founder of this journal and a great and influential personality [2, 3]. In addition, this short survey on the evolution of eukaryotes hopefully will stimulate future generations of researchers to find explanations and to test hypotheses for many still enigmatic phenomena of this fundamental part of biotic evolution on earth.

The starting point: prokaryotes

The evolutionarily oldest living cells are obviously prokaryotes. Such cells represent a membrane-surrounded containment of organic molecules with the ability of self-reproduction via DNA replication, transcription of DNA information into RNA, its translation into proteins, intracellular metabolic activity, and cell division, but without membrane-wrapped organelles. The genetic information is encoded within double-stranded circular DNA, the nucleoid, propagated through semiconservative and discontinuous replication, and retained intact by various DNA repair mechanisms. The origin of prokaryotes fades away in the dark age of the archaic earth.

The next step: unicellular eukaryotes

The first achievement of post-cellular evolution was the origination of unicellular eukaryotes. This step required several novel inventions. One was the *gain of a* '*true*' *nucleus*, separating the DNA from the cytoplasm with a double membrane, as the eponymous (name-giving) feature of eukaryotes (Fig. 1). Instead of being circular, the nuclear genome of eukaryotes consists usually of more than one linear DNA molecule, which form, mainly by means of basic histones and other proteins, hierarchically structured chromosomes which are superior to prokaryotic genomes (see below). The persistence of *linear chromosomes* in turn required the invention of telomeres and centromeres (Fig. 2).

Telomeres are formed by rather conserved terminal sequences. Telomere sequences, together with specific proteins, protect the ends of linear DNA helices from exonucleolytic digestion as well as from 'fusion' with each other via DNA repair proteins, which could mistake the ends as broken DNA double-strands. Telomeric sequences are usually added to the 3'-ends by the enzyme telomerase, as arrays

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This article is dedicated to commemorate the Birth Centenary of Prof. Arun Kumar Sharma.

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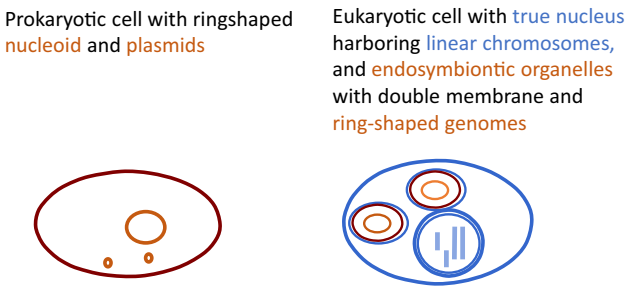

Fig. 1 Schemes of a prokaryotic and a eukaryotic cell

of 2 to > 20 nucleotides (complementary to its internal RNA template and specific for distinct phylogenetic groups), to the linear DNA ends in dividing cells. Telomerases apparently evolved from retroviral reverse transcriptases. A single-strand overhang, of the chromosomal telomere arrays can fold back, forming a tri-stranded structure, the so-called T-loop (Fig. 2). The T-loop is stabilized by telomeric proteins and prevents access of proteins which mediate exonucleolytic and DNA repair activities. At the same time telomeres solve the 'end-replication problem', which does not exist for circular genomes. All known DNA-polymerases can add nucleotides only in 5' to 3'-direction, and need an RNA primer sequence to start. After removal of the primer, there is no sequence at the ends to which nucleotides can be added. Therefore, each round of replication would led to a loss of nucleotides at both ends of the double strand, which is compensated for by telomerase activity.

Centromeres, like telomeres, are as old as linear chromosomes. They represent the binding site(s) on a chromosome for the so-called kinetochore protein complex at which the fibers of the spindle apparatus dock during nuclear division to pull the sister chromatids (containing the newly replicated identical double helices) to opposite spindle poles, ensuring that both daughter nuclei (and cells) obtain the same genetic material. DNA sequences of centromeres (and even the kinetochore proteins) are much more variable than those of telomeres. The evolutionary origin of centromeres is still awaiting its elucidation.

In eukaryotic cells, cell division is preceded by a nuclear division, called *mitosis*. During mitosis, after transient disassembly of the nuclear double membrane, identical sister chromatids first line up at the 'metaphase plate' in the middle of the cell and thereafter segregate to opposite poles. This process is mediated by the *spindle apparatus* (Fig. 2). The spindle apparatus is established by microtubule organizing centers. After poleward migration of the two centrosomes of the *centriole*, another organelle which newly appeared in eukaryotes and resembles the basal bodies forming flagella and cilia of motile eukaryotic cells, the microtubuli of the spindle apparatus are formed starting from centrosomes. The mitotic nuclear division guarantees equal distribution of linear sister chromatids to daughter cells, in contrast to the irregular segregation of plasmids, the facultative heredity substrates which occur in some prokaryotes in addition to their nucleoid. For early ideas about evolution of mitosis see [8].

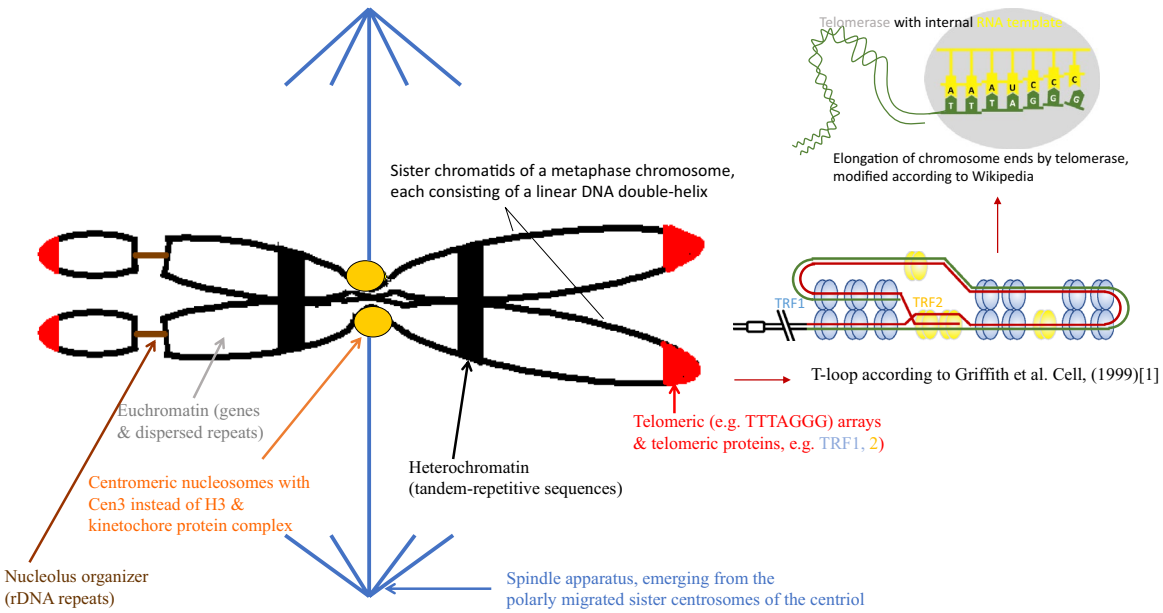

Fig. 2 Scheme of a replicated linear eukaryotic chromosome at mitotic metaphase. On the right below: terminal T-loop stucture; above: telomerase activity

Another novelty in eukaryotes is *sexuality* (Fig. 3). possibly derived from bacterial conjugation processes in combination with recombination repair of DNA double-strand breaks (DSBs). Sexuality provides an option to generate from two parental organisms a variety of progeny harboring parental alleles (variants of a gene) in different combination compared to their parents. Beginning with the fusion of two genetically similar but not identical parental cells, a diploid somatic cell, harboring homologous pairs of parental chromosomes, is generated. The diploid somatic cell undergoes *meiosis*, i.e. two nuclear divisions without DNA replication between both divisions. During the first division the replicated parental chromosomes, after scheduled induction of DSBs, form homologous pairs, mediated by cohesin protein complexes, and exchange alleles in the course of DSB repair, yielding so-called 'cross-overs'. Thereafter, the homologous chromosomes (instead of sister chromatids) segregate to opposite spindle poles, *reducing the chromosome constitution from diploid to haploid*. Although each daughter nucleus receives the same number of chromosomes, it is accidental which chromosome of a homologous pair segregates into which daughter nucleus. Cross-overs as well as accidental segregation of parental homologous chromosomes provide variable combinations of parental alleles in gametes. During the subsequent second meiotic division, similar as during mitotic divisions, the sister chromatids segregate into daughter nuclei. Thus, one diploid somatic cell generates four haploid nuclei. *Fusion of two haploid cells results again in a diploid cell* with two parental sets of chromosomes. Although deviations from sexuality via (transient, facultative) parthenogenesis may be adaptive to rapidly colonize new environments, sexual propagation is a primary feature of eukaryotes. The detailed evolution of sexuality, i.e., how the energetically expensive steps of the sexual cycle arose, is still a matter of speculation [e.g., 4, 10].

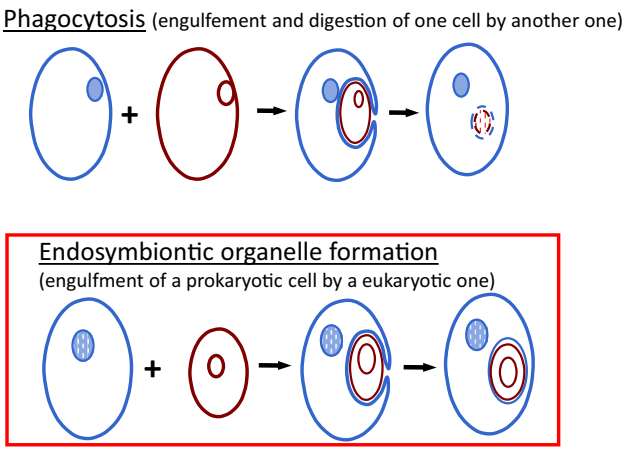

Fig. 4 Pathways of (non-sexual) cell fusion and their consequences, modified according to Schubert [10]

Other crucial inventions were the non-nuclear membrane-enclosed organelles, the *mitochondria* for generation of energy from nutrients and its storage as ATP, and the

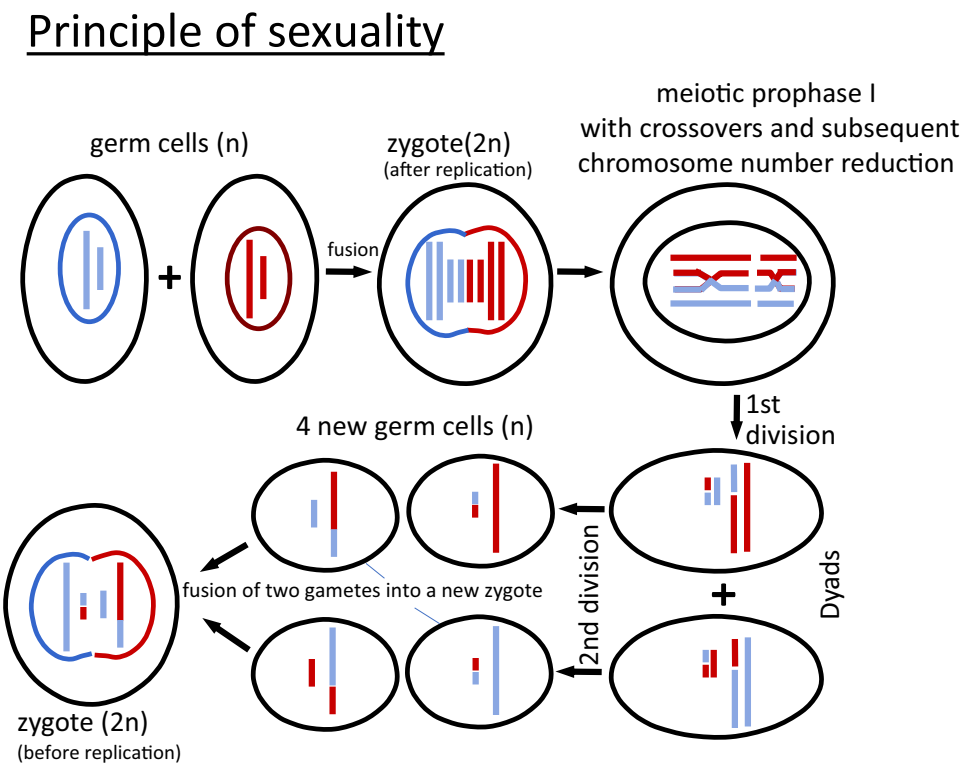

Fig. 3 Principle of sexuality, modified according to Schubert [10]

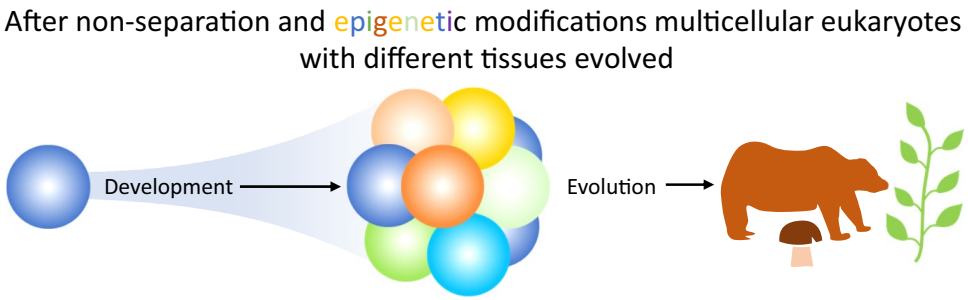

Fig. 5 Multicellularity of eukaryotes after non-separation of divided cells, epigenetic chromatin modification and differential gene expression; modified according to Márquez-Zacarías et al. [5]

plastids for photosynthesis. While mitochondria most likely occurred in all eukaryotes (a few apparently have lost them secondarily), plastids, occur in form of chloroplasts primarily in all green algae and vascular plants. Both organelles most likely *arose from* '*endosymbiosis*' [for review see 6], a process during which one cell is engulfed by another one. Instead of being digested, as during phagocytosis, the engulfed cells remained functional and multiply themselves at the benefit of the host cell which enslaved them (Fig. 4).

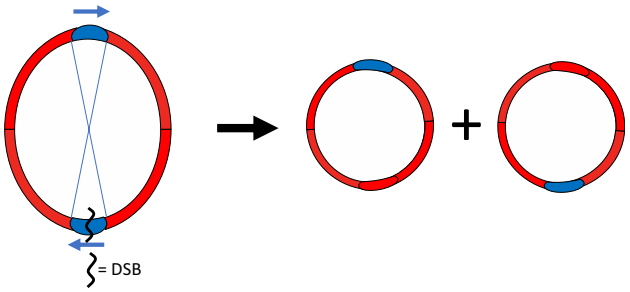

Fig. 6 Recombinative DSB repair using dispersed direct repeats (blue) as template may distroy circular prokaryotic genomes

Sequence comparison of the multiple ring-shaped organellar DNA molecules indicate that mitochondria are derived from proteobacteria and chloroplasts from cyanobacteria.

Remarkably, *cell fusion* is, aside of mutagenesis via mis-repair of DSBs (for review see [11]), an important feature generating the evolutionary novel quality of eukaryotic cells. Cell fusion may be *either irreversible* as in capturing prokaryotes which become mitochondria and plastids (Fig. 4 enframed), *or* at least *indirectly reversible* as during fusion of haploid gametes into diploid somatic cells [10] (Fig. 3). Whether also the eukaryotic nucleus is a product of endosymbiosis as speculated previously [7, 9] (for other references see [6]), or rather a product of intracellular compartmentation, is still a matter of debate.

The final step: multicellular eukaryotes

If mitotically *dividing cells do not separate but specialize* their function via *epigenetically* regulated chromatin modification and differential gene expression, multicellular organisms emerge with the possibility of further increasing polymorphism and adaptability compared to unicellular ones (Fig. 5). At the same time, *multicellularity is linked*

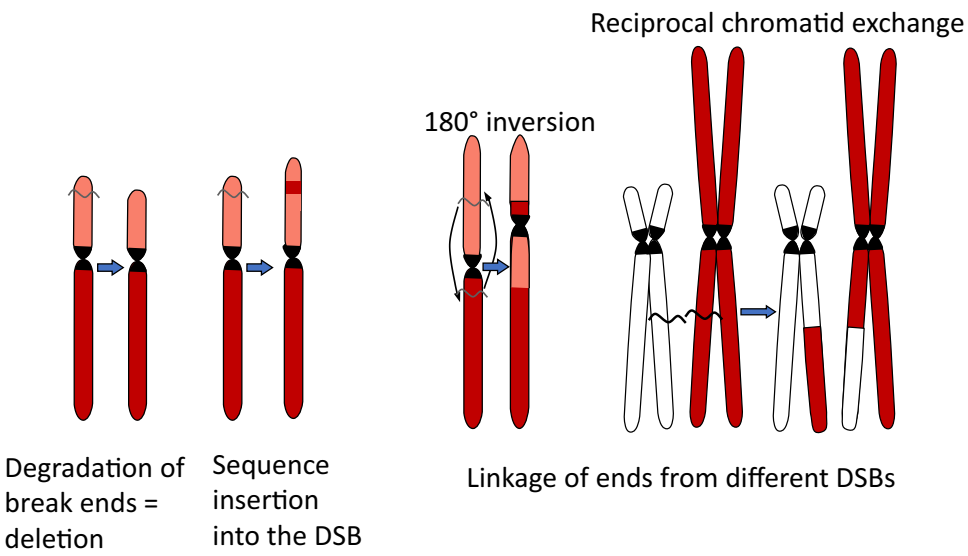

Fig. 7 Mis-repair of DNA double-strand breaks (DSBs) causes mutations

with developmentally fixed organismic mortality. On the other hand, due to formation of multiple germ cells, a multicellular eukaryote has the potential to produce a large number of progenies.

Benefits of eukaryotes by far outweigh their disadvantages compared to prokaryotes

In spite of the high energetic costs for meiosis I and for finding (in case of outbreeding) a complementary sexual partner, needed for fusion of haploid gametes into the next diploid generation, the eukaryotic status bears several advantages over the prokaryotic status. Circular prokaryotic chromosomes are restricted in size and tolerate little dispersed repetitive sequences. In case of breakage within in one of several dispersed repeats of the same orientation, recombination repair, using ectopic homologous sequences as a template, would destroy the circular chromosome by splitting it into two molecules (Fig. 6). Linear *eukaryotic chromosomes* show a much larger size tolerance and can potentially *accumulate* all types of *sequences*, if not immediately harmful. Sequence accumulation and repeat tolerance led to genome expansion and provided a play-ground for evolution [12], *enabling* the huge morphological and physiological *polymorphism* of-in particular multicellular—eukaryotes, as well as their enormous *adaptability* to changing environments. Adaptive polymorphism is further enhanced through variable allele combination via crossover and random segregation of parental homologous chromosomes during the first meiotic division as well as via fusion of haploid gametes from different parents. Moreover, the diploid somatic stage tolerates harmful recessive mutations which usually are sorted out via the haploid stage. The inevitable organismic *mortality* of multicellular eukaryotes is (over-)*compensated by* the potentially 'eternal' life via their *germline* (cells leading eventually to multiple gametes).

(Main) Drivers of eukaryotic evolution

Erroneous DSB repair (already present in prokaryotes) is the main source of genetic novelty as substrate for evolutionary selection (Fig. 7). Specific variants of DSB repair (emerging via mutations of repair components) can led to increasing (insertion bias, including retroelement spreading) or decreasing (deletion bias) genome size [12].

Multiple breakage and linkage of DNA ends from different breaks leads to genome rearrangement (Fig. 7 right part), e.g. genome differentiation via chromatin elimination, cell differentiation via gene rearrangement (immunoglobulin gene maturation) or de-differentiation (cancerogenesis), and even speciation via chromosome rearrangements that generate reproductive barriers by disturbing correct meiosis and thus decreasing fertility of heterozygotic individuals (for review: [11]).

Interspecific hybridization, via fusion of unreduced germ cells or via fusion of haploid gametes and subsequent chromosome doubling, leading to allopolyploidy, further increases genome size and adaptability, compensates for genome shrinkage and detrimental mutations, and offers the option of neofunctionalization of duplicated genes.

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Declarations

Competing interest The author declares: no competing interest and funding.

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