# BIOLOGY

## THE ORIGIN OF THE EUKARYOTIC CELL

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**ABSTRACT: Total Hfr cell conjugations can lead to diploidy and the authors hypothesize such a process could be involved in the origin of the eukaryotic nucleus. Total conjugations are uncommon and the diploidy they produce is transitory, nevertheless they do occur spontaneously in nature; something that does not occur with bacterial fusion as in "bacterial cannibalism" or "hypersex" presented by Margulis. The dilemma of accepting at least one instance (the one that originated the first diploid cell) in which the receptor bacteria conserved and integrated the transferred DNA exists in both theories. Implicating conjugation in the appearance of the eukaryotic nucleus has certain advantages. It provides an understandable explanation of the tendency to recover the diploid state after the first meiosis. The fact that this method of genetic exchange persists in the ciliates could be considered, to a certain degree, an argument in favor of total conjugation.**

### INTRODUCTION

Every living being is the expression of a formula written in its genetic inheritance in the form of a double strand of polynucleotides, which when separated has a tendency to recover its duplex structure. This tendency has served (and serves) prokaryotic life well, permitting it to easily create two daughter cells identical to the original progenitor cell. After monopolizing every expression of life on the planet for two billion years, suddenly (or appearing so due to our ignorance regarding the intermediate steps) more complex eukaryote cells appeared with duplicated DNA (haploid vs. diploid respectively) and a new method of reproduction. This new sexual reproduction results from the collaboration of two progenitor cells contributing to the genetic inheritance of their descendants. Through a process called meiosis each progenitor creates haploid gametes, which in turn fuse together during fertilization to recover the diploid state. How did this come about?

### THE ENDOSYMBIOTIC THEORY

In truth, we don't know. So we must delve into the murky waters of hypotheses, which always involve a factor of risk, but in this case even more so. In fact, the extended period of time that passed before the eukaryotic cell first appeared reflects the enormous difficulties that must have existed for the process to evolve. Furthermore, the difference between eukaryotes and prokaryotes doesn't end with diploidy and sexual reproduction, but includes several other characteristics. Knowing the order in which each one appears would be invaluably helpful but, unfortunately, they all showed up at once. Nevertheless, the fact that there are diploid cells lacking mitochondria (although we don't know if they've never had them or if they lost them subsequently) and others that do not engage in sexual reproduction (with the same caveat as with mitochondria) seems to support the thesis that diploidy was acquired prior to these other characteristics. If

this is the case, how did it come to be?

One of the most widely accepted theories is Margulis' endosymbiosis, in which she distinguishes between sex and hypersex. Bacterial sex is any mix of genes coming from more than one source and thus includes all types of genetic transfer occurring in bacteria. This may be the result of lysis (transformation), caused by a virus (transduction or transfection), or via a plasmid (including conjugation).

After bacterial sex, hypersex appeared, which is a "permanent symbiotic association that generates organisms with genes from different origins" and is often present in bacteria (almost always as the presumed victims of protozoan fagocytosis, ready to be digested, although in some cases they would have escaped that fate). Thus, eukaryotic cells have organelles (mitochondria and chloroplasts) due to the fact that hypersex allowed them to incorporate into their cytoplasm what were at one time independent and free-living bacteria. Margulis proposes that at some point certain bacteria imitated that behavior. One of them engulfed another by fagocytosis, but instead of digesting the engulfed cell's DNA, it was left intact resulting in a diploid cell: "Normally, bacteria never fuse; they make contact briefly to send genes in one direction from one cell to another. But, in hypersex they fuse forever […]. The first hypersexual fusion of bacteria – between an obscure type of walless microbe belonging to the archeobacteria and a walled swimer – led to the earliest of the nucleated cells..." (Margulis, L. & Sagan, D., 1997. What is sex? (p. 79). Nevraumont Publishing Company. New York).

Had things occurred in this manner, we would expect this "new species" to behave as all others. With each asexual division it would duplicate its DNA and divide it in equal parts, producing daughter cells that are also "diploid." That situation may have persisted for millions of years allowing for the other characteristic traits of eukaryotic cells to evolve, such as: the nuclear membrane, chromosomes, mitotic apparatus, mitosis, cytosqueleton, chloroplasts, mitochondria, etc. Until one day an anomaly occurred in the DNA replication giving rise to the first meiosis.

How did that happen? It was an accident, of course, with no teleological intention. The cellular division process involves many genes acting in sequence; each initiating its activity when the prior gene has completed its function. The gene that divides the DNA would begin its activity after the gene that duplicates the DNA had finished its task, until one day something activated it prematurely. When that anomaly occurred the process should have stopped, but since there were two homologous DNA chains present, the gene that divides the DNA was able to complete its task, dividing and distributing it into two almost identical haploid groups.

Because this process is so universal today, from the very beginning it must have established two overlapping divisions.
Something changed the normal rhythm and by moving the second process forward an atypical pattern arose (like an extra systole that creates an abnormal heartbeat by provoking a myocardial contraction out of sequence).
And that anomaly resulted in the division of the mother bacterial cell into four haploid daughter cells; the origin of future gametes.

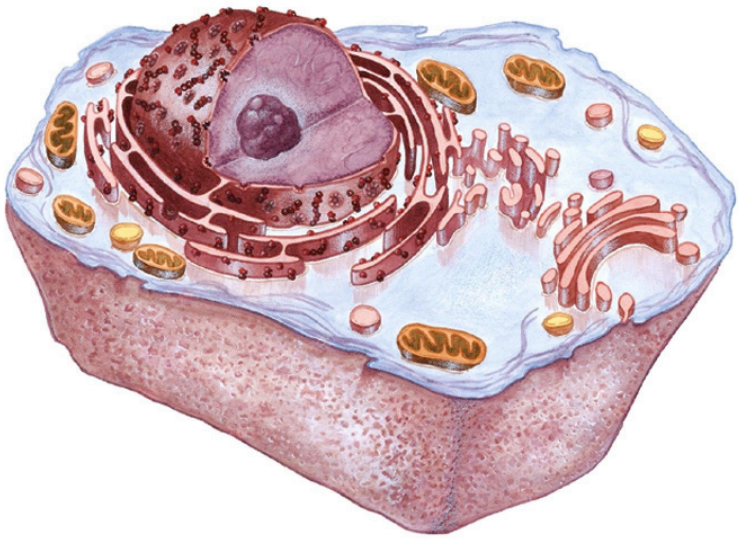
That would have been the end of the story, if those haploid cells had not fused with each other in a process that would later become the fusion of gametes. Margulis attributes this to the same endosymbiotic phenomena that, according to her, already existed in the origin of the eukaryotic cell (similar to co-generic cannibalism of certain protists under extreme conditions, specifically in the Trichonympha, a group of hypermastigotes studied by Cleveland. He believed that in some cases the process did not stop with the digestion of the fagocytosed cells chromosomes, but instead they remained intact to become part of the chromosomic legacy of the predator cell)..

## GRAY AREAS OF THIS THEORY

Endosymbiosis is almost unanimously accepted to explain the appearance of chloroplasts and mitochondria in eukaryotic cells, as well as other parts of the theory, because all the data support it. For example, both their DNA and rRNA 16S sequences differ from those found in the eukaryotic nucleus, and instead are similar to certain eubacteria. Furthermore, the exogenous origin of these organelles helps to explain the somewhat independent and autarkic behavior they have compared to the rest of the cell.

The possibility that endosymbiosis may have contributed to the formation of the eukaryotic nucleus is something quite different, on the other hand. Prestigious author (Nobel Laureate, 1974) Chrisitan de Duve calls this into question, "Endosymbiont adoption is often presented as resulting from some kind of encounter –agressive predation, peaceful invasion, mutually beneficial association or merger – between two typical prokaryotes. But these descriptions are troubling because modern bacteria do not exhibit such behavior." (de Duve, C., 1996). His article ends by emphasizing, "The adoption of endosymbionts undoubtedly played a critical role in the birth of eukaryotes. But this was not the key event. More significant (and requiring a much larger number of evolutionary innovations) was the long, mysterious process that made such acquisition possible: the slow conversion, over as long as one billion years or more, of a prokaryotic ancestor into a large phagocytic microbe possessing most attributes of modern eukaryotic cells."

It's possible that Christian de Duve is correct, and as far as we know, there is not a single documented case of such "bacterial fusion" as Margulis defends. The endosymbiotic origin of



mitochondria and chloroplasts seems to require a prior process in which a prokaryote became a cell capable of engulfing bodies with the volume of bacteria. In fact, the ability to carry out phagocytosis presumes changes to the cell wall, an increase in celular volume and the presence of a cytosqueletal and reticulate structure (Cavalier-Smith). Then, the question is whether diploidy was also among those attributes already aquired by that procaryotic cell. It would be difficult for that cell to aquire these other traits without a parallel increase of the genophore DNA; perhaps even to the point of diploidy.

It could be argued that, although not a single case of bacterial endosymbiosis has been proven, it is a possibility that should not be rejected outright. There have been such different environmental conditions in the past that we can't rule out the possibility that some bacteria might have undergone such a process. And we have to explain, one way or another, both the duplication of the DNA in the eukaryotic cell as well as the mixture of archeobacterial and eubacterial genes found by several authors (Gupta, Lake, Doolittle, Rivera, and others). However, how a phenomenon that took more than a billion years to occur then becomes commonplace in gametes is left unexplained. Is it possible to believe, as Margulis would suggest, that it is due to the same process? And if so, why such a different behavior? Why has something that might be considered nearly a miracle (considering the time required for it to occur) become a common fact? Why do gametes behave so differently than all other haploid cells? Why do they even differ among themselves in behavior, some having the tendency to "phagocytose" (or allow themselves to be penetrated), while others to be "phagocytosed" (or penetrate)?

Without denying the importance of endosymbiosis in the origin of chloroplasts and mitochondria, perhaps other processes have been involved in the evolution of the diploide cell and can help us answer these questions.

## CONJUGATION

Besides sexual reproduction, there are other processes – referred to as bacterial sex by Margulis – by which bacteria mix their DNA. One of the most common of these is conjugation, which is capable of producing more or less fleeting states of diploidy. "A diploid state can arise transiently in bacteria, as a result of genetic transfer, but full diploidy is rarly attained…" (Stanier, R., Ingraham, J., Wheelis, M. & Painter, P., 1986). This process involves two bacteria that approach each other, one of them transfering its genes to the other. The comparison with males and females of sexual species is even greater considering there are cases in which the donor bacteria possess tube-like structures that function similarly to copulatory organs. This behavior is determined by the presence of a sexual factor, or the "F factor": a formation that is present, either integrated into the genophore (Hfr bacteria), or free in the cytoplasm (F+ or "F positive" bacteria). Those bacteria that do not possess this sexual factor (F- or "F negative" bacteria) always act as receptors during the transfer.

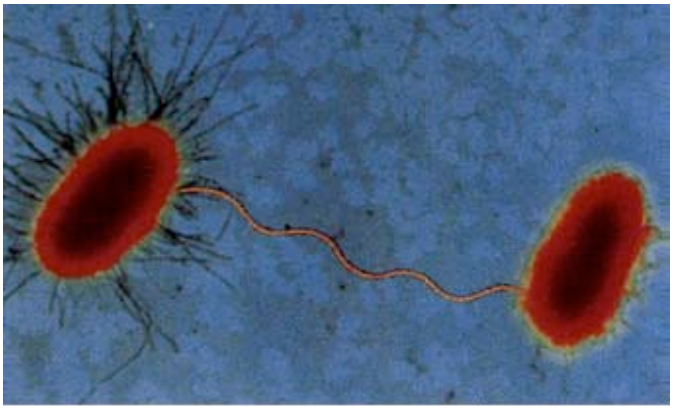
Regardless of where the F factor is located, during conjugation it separates its DNA strands and initiates the transfer of one copy to the receptor cell. Often there is complete transfer of the F factor from the F+ bacterium (it might be the only DNA passed between bacteria) and, once the complimentary strand is synthesized, the receptor cell also becomes an F+ bacterium. But sometimes the transmission can include another gene (sexduction). The location of the F factor can vary over time: if the F factor is free in the protoplasm ("F+" bacterium) and at some point in time becomes incorporated into the genophore, it will then be replicated and transmitted to the daughter cells of what is now an "Hfr" bacterium. However, if the opposite occurs and the F factor detaches from the genophore and becomes free floating in the protoplasm it can carry some genes from the bacterial DNA along with it. This "combined" F factor is noted as F' (F-prime). When F' bacteria engage in conjugation, the combined genes can be transfered to the receptor bacterium.

Upon initiating conjugation, the F factor in Hfr bacteria pulls along the DNA in which it is inserted, but the strand of genes is too long and the pilli maintaining the two bacteria united tends to separate before the transfer is complete. Since the point in the DNA where conjugation begins rarely coincides with the F factor insertion points in the genophore, but instead tends to occur at an intermediate site, the transfer includes a fragment of the F factor together with some bacterial DNA. The receptor bacterium will continue to be F- because the F factor must be nearly complete to carry out its function. Nevertheless, and although it occurs only now and again, there can be complete conjugation whereby the donor bacterium transfers the F factor along with one complete strand of DNA. Thus the receptor bacterium becomes diploid, although briefly.

At some point in time, one of those Hfr cells permanently integrated the F factor into its genophore and its pilli became more resistant. Thus all of its future conjugations were complete, transferring the complete F factor along with one of the genophore strands. There was at least one time, – specifically following conjugation between an archeobacterium and a eubacterium – in which the receptor conserved the donor DNA, becoming an Hfr bacterium with twice as much genetic material (diploid).

## POSSIBLE EVOLUTIONARY SIGNIFICANCE

None of the research demonstrating the presence of Archeabacterial and Eubacterial genes in the eukaryotic nucleus (Gupta, Lake, Doolittle) present, in and of itself, an argument in favor of the endosymbiotic theory. They prove the existence of



that genetic mix, but they do not help to decipher the way in which that mix came about. Conjugation, even complete conjugation, is more frequent in bacteria than the alleged cellular fusion, which never occurs. Other than that detail, the new hypothesis offers no advantages, nor disadvantages, over the previous one (everything said for the one is valid for the other) until we consider the first meiosis.

That new bacterium and its descendants would reproduce asexually. To do so, it would duplicate its DNA and divide it among its "diploid" daughter cells, which would be capable of carrying out conjugation, although the placement of the F factor on the genophore would facilitate the appearance of regulatory mechanisms placing conditions on these cells. Most structural genes, if not all have these mechanisms and once the F factor was integrated into the genophore it too would become a structural gene. This situation would present challenges that bacteria would have to overcome. At first they would obey their older genes and disregard the new ones. However, doubling the amount of DNA would double the number of possible mutations that would appear on either DNA strand, which in turn would facilitate the formation of distinct alleles at each locus. Over time, some bacteria would take advantage of the neglected genes if the result was advantageous and the double DNA would stimulate improvements. The duplication of the DNA should also help bring about an increase of celular volume. Thus the first step would have been achieved towards that phagocytic cell of which Christian de Duve speaks, which in theory requires a larger size than its potential prey. The remaining evolutionary conquests could be added later: the internal skeleton for support, a flexible membrane capable of engulfing extracellular objects (and since it could fold, it would increase the available exterior surface and thus facilitate the possibility of achieving even greater celular dimensions), a network of compartments available to digest the prey, a nuclear membrane, organization of chromosomes (it would already have enough genetic material available for at least two), centromeres, the necessary structure to initiate mitosis, etc. Then the first meiosis occurred, which (based on the importance it now has) couldn't be due to a functional error, but brought about by a mutation capable of being transmitted to the daughter cells. Through a series of circumstances, meiosis became such a decisive event that it converted that first cell into the mother of all eukaryotes, including humans. Based on the uniformity of this trait, it is possible that all eukaryotic beings are the descendents of the first cell to undergo meiosis. What were the many factors that so favored this trait in the course of its evolution?

## THE ARRIVAL OF SEX

The most important is the presence of our illustrious F factor. The F factor included on the DNA strand, which at one point was extrinsic DNA, would be replicated in the first division and passed on to the two resulting "Hfr" nuclei. However, during the second division it would only be passed on to two of the four daughter cells, those receiving the DNA strand with the F factor integrated. The process would give rise to four haploid cells from the primitive diploid mother cell. However these daughter cells would not be identical, contrary to the other theory. Two would be F- and two would be Hfr (similar to those cells that gave rise to the first diploid cell through complete conjugation). These cells could immediately procede to a double conjugation resulting in two diploid Hfr cells identical to the mother cell that initiated the process and two Hfr haploids, or all the daughter cells could remain in their haploid state reproducing asexually (remember we argued previously that regulatory mechanisms would have occurred). Here we are entering the world of the ciliates, the only eukaryotes that depend on complete conjugation to exchange their genetic material. Just as a diploid cell gave rise to four haploid daughter cells through the first meiosis, the diploid ciliate micronucleus also divides into four haploid micronuclei. Following that micronuclear division there is no division of the protoplasm, instead three micronuclei degrade (something very similar to the formation of ovocytes in many species where three polar bodies are formed and eliminated) and the fourth one divides again to form a mobile micronucleus, which will be passed to the conjugating cell, and a sedentary micronucleus, which will fuse with the mobile micronucleus of another cell. At some point in time the shift from conjugation to fusion occurred. For the purpose of transmitting genetic code, both processes are equivalent. The difference is that with conjugation the donor cell continues to exist independently. This would appear to be beneficial as it doubles the number of organisms in each generation; but this might be a premature conclusion. To restore the duplex form, the conjugating cells have to synthesize the complimentary strands of DNA; the donor strand they received as well as the strand they retain during conjugation. In addition to the diploid state, they also have a greater protoplasmic volume, and these two requirements can be catastrophic in a depleated environment. Thus in certain cases it could have been more beneficial to fuse the genophores and their corresponding cytoplasm. It is not difficult to imagine a mutation leading to the first fusion and selection supporting that trait. Over time that behavior branched into three distinct forms: asexual reproduction remained both in the diploid and the haploid states, asexual reproduction was restricted to the diploid state, or it was reserved for the haploid state.

In the first of these cases we are talking about the haplo-diplobiontic life cycle of yeast (S. cerevisiae). These cells grow in their diploid state and reproduce asexually in a rich environment.

But if the environment becomes depleated, they go through a meiotic process giving rise to four haploid cells of opposite mating strains (two of each type), which can continue dividing asexually or fuse to recreate their diploid form.

The second case is similar to the diplobiontic cycle of certain Heliozoa (Actinophrys), which divide in a way that produces two daughter cells identical to the mother cell. Each of these cells then undergoes a process of DNA reduction by eliminating one of the polar bodies, becoming haploid hologamic gametes with identical cellular configuration, which fuse with each other (pedogamy).

In the third case we are approaching the haplobiontic cycle as seen in Chlamydomonas, whose haploid state can reproduce asexually for a prolonged period without losing the potential to behave as a gamete, fusing with each other to create diploid zygotes. These zygotes immediately undergo meiosis, giving rise to four haploid daughter cells.

New influencing factors appearing later could have shifted the F factor to a “Y” chromosome, which accentuated sexual bipolarity. This would be exemplified by the anisogamy present in some protozoan species. During one of the stages mentioned, endosymbiosis must have occurred giving rise to the mitochondria, chloroplasts and perhaps peroxisomes.

In principle all of this commotion doesn’t appear to have much value. But over time its evolutionary advantages have become evident. Asexual division allowed each strain to continue unchanged until a new mutation occurred. The rate of new mutations for each gene is estimated to be on the order of 10-8 for each generation. Therefore in 100 million generations, or thousands of years with adequate environmental nutrients, each gene could have undergone a mutation. To be useful, there had to be correlated mutations within the same strain, something that is extremely difficult. The new “reproductive” process brought about an amazing revolution. It allowed for “incest” between sister cells, without making it compulsory; thus creating a fortunate opportunity for mixing genes, potentiating differences and expressing them more quickly. Extremely rare coincidences within the same strain were no longer necessary as they could be found in the millions of genetic combinations that would come about on an hourly basis. It is true that the variants were packaged inside the DNA strands and this phenomena didn’t achieve its full potential until chromosome crossover began to occur. Nevertheless, compared to what existed previously, this evolutionary jump would have been exceptional and the drastic appearance of new forms of life that occurred thereafter seems to bear witness to its success.

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