

Prokaryotic and eukaryotic chromosomes: what's the difference?

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Summary

It is widely held that the profound differences in cellular architecture between prokaryotes and eukaryotes, in particular the housing of eukaryotic chromosomes within a nuclear membrane, also extends to the properties of their chromosomes. When chromosomal multiplicity, ploidy, linearity, transcriptional silencing, partitioning, and packaging are considered, no consistent association is found between any of these properties and the presence or absence of a nuclear membrane. Some of the perceived differences can be attributed to cytological limitations imposed by the small size of bacterial nucleoids and the arbitrary choice of representative organisms for comparison. We suggest that the criterion of nucleosome-based packaging of chromosomal DNA may be more useful than the prokaryote/eukaryote dichotomy for inferring the broadest phylogenetic relationships among organisms. *BioEssays* 22:481–486, 2000.

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Introduction

It has long been customary to divide organisms into two groups, the prokaryotes, whose DNA intimately interacts with cytoplasm, and the eukaryotes, whose DNA is separated from cytoplasm by a nuclear membrane. Eukaryotic chromosomes generally change from a diffuse form to a highly condensed one during mitosis, and chromosomal DNA, which has specialized ends (telomeres), is usually packaged into nucleosomes by histones. Ploidy levels can differ widely, and chromosomal regions, or even whole chromosomes, can be silenced by compacting parts of the genome through heterochromatinization. In contrast, prokaryotic chromosomes have often been thought to lack those properties; thus, it has been convenient to relate the differences to the presence or absence of a nuclear membrane. Recent studies, however, reveal that prokaryotic chromosomes, or at least portions of them, undergo large movements similar to those found in eukaryotic mitosis (reviewed in Refs. 1,2); moreover, examples of histone-based DNA packaging have

been found in some prokaryotes (reviewed in Refs. 3,4). By blurring the distinction between eukaryotic and prokaryotic chromosomes, these observations raise the possibility that the two groups might not differ categorically in terms of other chromosomal features.

To examine distinctions between prokaryotic and eukaryotic chromosomes we have surveyed a large number of species. We found so many exceptions to commonly held views about chromosome multiplicity, ploidy, linearity, heterochromatinization, partitioning, and histone-based DNA packaging that we were forced to conclude that chromosomal properties do not correlate well with the presence or absence of a nuclear membrane. The fading prokaryotic/eukaryotic dichotomy means that some other character(s) should be used if we are to divide organisms into two groups. DNA packaging strategies could serve as such a character. Identifying homologous features of chromosome structure and function among organisms would then take on new meaning. Below, we begin an argument for a nucleosomal/non-nucleosomal dichotomy by pointing out counter-examples to traditional prokaryotic/eukaryotic distinctions (summarized in Table 1).

Chromosome number and genome size

Prokaryotes are frequently thought to contain only a single chromosome. Small size, however, has usually precluded a direct count of chromosomes in prokaryotic cells. Recently, chromosome number has been inferred from DNA fragment mapping and pulsed-field gel electrophoresis, techniques that have generated a growing list of species with two or more chromosomes (in addition to very large plasmids). The number of large, circular-mapping chromosomes is 2 for *Vibrio* species,^(5,6) *Deinococcus radiodurans*,⁽⁷⁾ *Rhodobacter sphaeroides*, *Leptospira interrogans*, and *Brucella* species, three for *Rhizobium meliloti* (Ref. 8 and references therein), and 2–4 among isolates of *Burkholderia (Pseudomonas) cepacia*.⁽⁹⁾ Some *Agrobacterium* species contain one circular- and one linear-mapping chromosome,⁽⁸⁾ and *Paracoccus denitrificans* contains three unmapped chromosomes.⁽¹⁰⁾ The chromosome number in prokaryotes can even exceed that in eukaryotes, since the number is only one ($2n = 2$) in the ant *Myrmecia pilosula*.⁽¹¹⁾

Prokaryotes can also have a larger genome size. For example, the genomes of the prokaryotes *Myxococcus*

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Table 1. Counter-Examples of Properties Traditionally Associated with the Presence of a Nuclear Membrane

| Multiple chromosomes | | |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|------------------------------|
| Bacteria with two or more chromosomes: | | |
| <i>Vibrio</i> species | <i>Brucella</i> species | |
| <i>Deinococcus radiodurans</i> | <i>Rhizobium melliloti</i> | |
| <i>Rhodobacter sphaeroides</i> | <i>Burkholderia cepacia</i> | |
| <i>Leptospira interrogans</i> | <i>Paracoccus denitrificans</i> | |
| Eukaryotes with only one chromosome: | | |
| <i>Myrmecia pilosula</i> | | |
| Large genomes | | |
| Bacterial genomes (<i>Myxococcus xanthus</i> ; <i>Calothrix</i> strains) can be large than those of some eukaryotes (<i>Encephalitozoon</i> species; <i>Pneumocystis carinii</i>) | | |
| Polyploidy | | |
| Polyploidy in bacteria: | | |
| <i>Escherichia coli</i> | <i>Azotobacter vinlandii</i> | |
| <i>Methanococcus jannaschii</i> | <i>Borrelia hermsii</i> | |
| <i>Deinococcus radiodurans</i> | <i>Synechococcus</i> PCC 6301 | |
| <i>Desulfovibrio gigas</i> | <i>Epulopiscium fishelsoni</i> | |
| Linear chromosomes | | |
| Bacteria with linear chromosomes: | | |
| <i>Borrelia burgdorferi</i> ^a | <i>Agrobacterium tumefaciens</i> C58 | |
| <i>Rhodococcus fasciens</i> | <i>Streptomyces</i> species ^a | |
| Eukaryotes with circular chromosomes: | | |
| <i>Schizosaccharomyces pombe</i> (telomerase-deficient cells) | | |
| Transcriptional silencing, heterochromatinization | | |
| Prokaryotic silencing: | | |
| <i>E. coli</i> plasmids (regions of P1 and F) | | |
| <i>Bacillus</i> species (spore) | | |
| <i>Chlamydia trachomatis</i> (extracellular elementary body) | | |
| <i>Halobacterium salinarium</i> (late exponential growth) | | |
| <i>Caulobacter crescentus</i> (swarmer cell) | | |
| <i>Epulopiscium fishelsoni</i> (condensed nucleoid) | | |
| Forms of Eukaryotic Mitosis | | |
| Undetectable metaphase plate: | | |
| <i>Saprolegnia ferax</i> | <i>Nanochlorum eucaryotum</i> | |
| <i>Aggregata eberthi</i> | <i>Aulacantha scolymantha</i> | |
| Undetectable chromosomal fibers: | | |
| <i>N. eucaryotum</i> | hypermastigids ^b | |
| <i>A. scolymantha</i> ^b | dinoflagellates ^{b,c} | |
| tricomonads ^b | | |
| Undetectable spindle pole structures: | | |
| <i>A. scolymantha</i> | higher plants | |
| <i>Trypanosoma</i> | many Protozoa | |
| Chromosomes permanently condensed: | | |
| euglenoids | hypermastigids ^c | dinoflagellates ^d |
| Chromosomes permanently decondensed: | | |
| <i>Zygorhynchus moelleri</i> | <i>N. eucaryotum</i> | |
| Binary nuclear division: | | |
| <i>N. eucaryotum</i> | <i>A. scolymantha</i> | |
| Histones and Nucleosomes | | |
| Eukaryotes with no histones or nucleosomes: | | |
| many dinoflagellates | <i>N. eucaryotum</i> | |
| Prokaryotes with histones and nucleosomes: | | |
| <i>Methanothermus fervidus</i> | <i>Methanobacterium thermoautotrophicum</i> | |

^aTelomeric ends on DNA.

^bSpindle fibers are present but may not directly move chromosomes.

^cSome species.

^dMost species.

xanthus (9.5 Mb of DNA⁽¹²⁾) and *Calothrix* strains (12 Mb;⁽¹³⁾) are larger than those of the eukaryotes *Encephalitozoon* species (2.3 and 2.9 Mb⁽¹⁴⁾) and *Pneumocystis carinii* (8.8 Mb⁽¹⁵⁾). Assuming a density of one open reading frame per 1.1 kb of prokaryotic chromosomal DNA, the number of different genes in *M. xanthus* and *Calothrix* exceeds the 5900 in baker's yeast.⁽¹⁶⁾ It is evident that neither chromosome number nor genome size (nor probably gene number) strictly correlates with the presence or absence of a nuclear membrane.

Chromosome copy number

Metazoans and plants are typically diploid, although ploidy can exceed 500 in certain tissues such as salivary glands in some insects and endosperm in maize.⁽¹⁷⁾ In contrast, prokaryotes are thought to be genetically monoploid. The copy number of genomic DNA can be higher in prokaryotes, however, than in most animal and plant tissues. For example, the number of genome equivalents per *Escherichia coli* cell is about 11 (the range is about 5–18) during early exponential growth in a rich medium and 2, 4, or 8 during stationary phase.⁽¹⁸⁾ The average chromosomal copy number for *Methanococcus jannaschii* is about seven (the range is about 3–15) during exponential growth and about three (range 1–5) in stationary phase.⁽¹⁹⁾ *D. radiodurans* is similar: the chromosomal copy number is 10 during exponential growth and four during stationary phase.⁽²⁰⁾ Copy numbers can reach about eight (the range is 3–18) for the cyanobacterium *Synechococcus* PCC 6301,⁽²¹⁾ 9–17 for *Desulfovibrio gigas*⁽²²⁾ and 16 for *Borrelia hermsii*.⁽²³⁾ For *Azotobacter vinlandii*, the copy number can increase from 4 to 40 to >100 as the culture progresses from early exponential through late exponential to stationary phase.⁽²⁴⁾ The most dramatic case among either prokaryotes or eukaryotes occurs in the eubacterium *Epulopiscium fishelsoni*: the DNA content varies by 4 to 5 orders of magnitude among individuals at different stages of the life cycle, and it can greatly exceed the amount of DNA found in mammalian nuclei.⁽²⁵⁾ These examples show that high genome copy number and variable ploidy among individual cells are not restricted to eukaryotes. Indeed, these properties might even be more common in prokaryotes but the monoploid nature of prokaryotic genetics in model organisms has hidden them from us.

Circular or linear form and telomeres

The famous autoradiographic image presented by Cairns,⁽²⁶⁾ which shows a circular replicating *E. coli* chromosome, appeared to confirm earlier genetic mapping data in this species and provided the foundation for the belief that prokaryotic chromosomes, being circles, are fundamentally different from the linear structures found in eukaryotes. Linear chromosomes, however, can occur in bacteria. Species with linear chromosomes include *Borrelia burgdor-*

feri, *Rhodococcus fasciens*, *Agrobacterium tumefaciens* C58, and *Streptomyces* species (Ref. 8 and references therein). Moreover, the ends of these bacterial chromosomes resemble eukaryotic chromosomal telomeres, structures that are designed to solve the “ends problem” of linear DNA replication (Ref. 27 and references therein). For example, chromosomal ends in *B. burgdorferi* are hairpin structures. In *Streptomyces*, the ends contain several short, inverted, repetitive sequences capable of forming hairpins, and attached to the 5' termini are proteins that probably prime synthesis complementary to the 3' ends of the DNA. Thus, not all bacteria have circular chromosomes. Since so few species have been analyzed adequately, it is not even clear that the circle is the most common chromosomal form among prokaryotes. Furthermore, the evidence for circularity for most bacterial chromosomes is based exclusively on mapping data, which alone cannot distinguish between circular and linear chromosomes (for example, the circular map of bacteriophage T4 DNA arises from circular permutation of a linear DNA). Even the circular form found by Cairns may not be representative, since nearly all of the molecules revealed by his technique were uninterpretable tangles and linear forms.⁽²⁸⁾

In eukaryotic cells, chromosomes are thought to be linear. However, exclusively circular forms can be maintained under certain conditions: the most common survivors among telomerase-deficient cells of fission yeast circularized their chromosomes.⁽²⁹⁾ Thus the circular/linear form of chromosomes does not necessarily correlate with the prokaryotic/eukaryotic form of cellular architecture.

Transcriptional silencing and heterochromatin

Not all genetic information carried by an organism is used continuously throughout its life cycle, and to ensure inactivation, some genomic regions are transcriptionally silenced by compaction with proteins. Eukaryotic silencing refers to the blocking of gene expression that spreads from a regulatory site on DNA, the silencer.⁽³⁰⁾ Silencing may be synonymous with formation of highly compacted heterochromatin.⁽³⁰⁾ Silencing also occurs in *E. coli*, spreading over a distance of about 10 kb from the *parS* site on plasmid P1⁽³¹⁾ and the *sopC* site on the F plasmid.⁽³²⁾ As in eukaryotes, silencing in bacteria requires particular proteins (ParB and SopB for these cases in *E. coli*).

A compacted DNA-protein complex can be recognized cytologically if it is large enough to bind sufficient Giemsa, Feulgen or other cytological stain. Such is the case in eukaryotes having large nuclear genomes in which silenced DNA can be recognized as heterochromatin, either as parts of chromosomes or as whole, compacted chromosomes (“B” chromosomes, for example; see Ref. 17). In some organisms, however, such as baker’s yeast and prokaryotes, the

genome is too small or there is too little silenced DNA for detection by staining in situ. Nevertheless, a general shut-down of genomic activity that is correlated with chromosomal DNA compaction does occur in bacteria. For example, during sporulation in *Bacillus* the chromosome is bound with new proteins as its transcriptional activity ceases.⁽³³⁾ Similarly, a histone H1-like protein in *Chlamydia trachomatis* probably causes chromosomal condensation during the conversion of the metabolically-active reticulate body to the inactive, extracellular elementary body form (Ref. 34 and references therein). A third case is observed when the cells of the archaeobacterium *Halobacterium salinarum* progress from early to late exponential phase of growth. The nucleoid changes from a form containing a naked DNA to one having a beads-on-string appearance typical of nucleosomal DNA; this change is also reflected in nucleoid sedimentation properties.⁽³⁵⁾ In another example, formation of swarmer cells of *Caulobacter crescentus* is associated with cessation of DNA replication, and the nucleoid appears to be in a compacted form when analyzed by sedimentation and electron microscopy.⁽³⁶⁾ After differentiation from the swarmer to the stalked cell form, DNA replication begins, and the nucleoid changes to a more open structure, possibly reflecting an activation of the genome. Finally, fluorescence measurements of DNA and RNA within enormous prokaryotic cells (up to 500 microns long) of *E. fishelsoni* suggest that decondensation and dispersion of the nucleoid is accompanied by increased transcriptional activity.⁽²⁵⁾ In their condensed state, these nucleoids resemble condensed eukaryotic chromosomes of dinoflagellates. Thus both prokaryotes and eukaryotes employ protein-mediated compaction strategies to activate and inactivate DNA. The apparent differences may be due to limitations of cytological analysis. It will now be interesting to look for homologies among the proteins involved. Such homologies, if found, could support the notion that chromosome structure represents a deeper evolutionary dichotomy than the presence or absence of nuclear membranes.

Forms of mitosis

The standard mitosis so familiar to most of us includes condensation and alignment of chromosomes at a metaphase plate, the movement of chromosomes to cell poles along spindle fibers emanating from centrioles or other structures at the spindle poles, and finally chromosomal decondensation after cytokinesis. Mitosis is enormously diverse among eukaryotes, however. For example, in *Saprolegnia ferax*, an oomycete fungus, and *Aggregata eberthi*, a member of the Sporozan class of Protozoa, mitosis is accomplished without the chromosomes appearing at a typical metaphase plate.^(37,38) For *Aulacantha scolymantha*, a member of the Rhizopoda, the chromosomes also move to the poles without prior arrangement at a metaphase plate,

and they do so without recognizable spindle fiber attachments to the chromosomes or spindle pole structures.^(38,39) Indeed, the absence of structure at the spindle poles is a feature of many Protozoa, including the flagellate *Trypanosoma*.⁽³⁸⁾ It is also a feature of higher plants.⁽⁴⁰⁾ In trichomonads, hypermastigids, and some dinoflagellates, microtubules participate in nuclear division, but they do not appear to be connected directly to the chromosomes.^(38,39) Instead, microtubules are attached to the outside of the nuclear membrane at the points where the chromosomal kinetochores are permanently attached to the inside of that membrane.^(38,39)

The chromosomes of some eukaryotes also fail to undergo condensation-decondensation during the cell cycle. For example, chromosomes are permanently condensed in most dinoflagellates, euglenoids, and some hypermastigids.^(41,42) Conversely, chromosomes are diffuse throughout nuclear division in the fungus *Zygorhynchus moelleri*⁽⁴³⁾ and the green alga *Nanochlorum eucaryotum*.⁽⁴⁴⁾ In the case of *N. eucaryotum*, the nucleus divides by simply pinching in two. No spindle fibers are seen. Indeed, the binary division of the nucleus in *N. eucaryotum* and in *A. scolymantha* more closely resembles the binary division of the enormous prokaryotic nucleoid of *E. fishelsoni* than the mitosis found in higher animals and plants. In view of such mechanistic diversity, the choice of a “standard” form of eukaryotic mitosis is clearly arbitrary, making it unclear which system to choose in order to contrast eukaryotes with prokaryotes. Until recently, lack of clear choice did not matter, since a process comparable to mitosis was unknown in bacteria.

We now realize that the failure to detect mitotic-like events has been due to cytological limitations imposed by small cell size: forms of mitosis have been demonstrated in *B. subtilis*, *E. coli*, and *C. crescentus* using fluorescence microscopy.^(1,2,45) The key observation is the rapid, cell cycle-dependent movement of the replication origin (*oriC*) region of chromosomes to opposite poles of the cell. Moreover, a region near the terminus of replication moves from a polar to a midcell position. To facilitate *oriC* movement, the bacterial chromosome appears to have both partitioning regions near *oriC* and proteins that bind to these regions. In *B. subtilis*, ten related, 8 bp inverted repeat DNA sequences are scattered across about 800 kb of the 4200 kb chromosome; eight of these are bound in vivo to Spo0J, a protein involved in partitioning chromosomes to daughter cells.⁽⁴⁶⁾ Such a distribution of bacterial centromere-like DNA elements is similar to the most common type of distribution of functional centromeric DNA in eukaryotes, the *CEN*-containing regional centromere.⁽⁴⁷⁾ Although additional work is needed to determine whether prokaryotes generally have a regional centromere or a point centromere (about 200 bp, found thus far among eukaryotes only in budding yeasts⁽⁴⁷⁾), it is apparent that at least some prokaryotic centromeres

structurally resemble eukaryotic centromeres. Indeed, it is difficult to explain chromosomal segregation in bacterial species that have more than one chromosome without invoking a mitotic-like process.⁽¹⁾

Microtubule-containing fibers have not been found attached to bacterial chromosomes, thus distinguishing chromosome partitioning in prokaryotes from that in most eukaryotes. It is possible, however, that the movement of bacterial chromosomes instead requires attachment to a movable membrane,⁽⁴⁸⁾ as proposed for chromosomal propulsion in closed extranuclear pleuromitosis.⁽³⁹⁾ The bacterial process might then be homologous to one of the six types of eukaryotic mitosis.⁽³⁹⁾ Many more experiments are required to develop this idea, since the possibility still exists that the force moving bacterial chromosomes is provided by the activity of DNA polymerase⁽⁴⁵⁾ rather than by a post-replicative process as observed for eukaryotes. It is also possible that many types of bacterial “mitosis” exist, with the consequence that homology searches among the mitosis proteins may be difficult.

Histones, nucleosomes, and chromosomal compaction

Chromosomal compaction in most eukaryotes involves the wrapping of DNA around histones to form nucleosomes. This is not true for all eukaryotes, however. Despite their permanently condensed chromosomes, many dinoflagellates contain neither histones nor nucleosomes.⁽⁴²⁾ Similarly, neither histones nor nucleosomes are detectable in the green alga *N. eucaryotum*.⁽⁴⁴⁾ With respect to prokaryotes, some (but not all) archaeobacteria contain histones that wrap DNA into structures that visibly resemble nucleosomes.^(3,49) Other prokaryotes, such as *E. coli*, have neither histones nor nucleosomes, although they do contain basic proteins such as HU and IHF that bend DNA sharply (wrapping of DNA within eubacteria is still being investigated). The smooth, extended non-nucleosomal appearance of DNA fibers from these bacteria shows that the forces that compact DNA in vivo are not maintained after extraction. The 1100 micron-long chromosome from *E. coli* in Cairns’⁽²⁶⁾ image also illustrates this point, since it was prepared in the absence of detergent or protease. In summary, examples exist for both prokaryotes and eukaryotes in which histones compact DNA into nucleosomes and examples in which both histones and nucleosomes are absent.

For non-nucleosomal organisms, the central question concerns how their DNA is compacted. An attractive idea is that compacting forces arise through macromolecular crowding due to a high cytoplasmic concentration of non-DNA-binding proteins and other large molecules, as shown in vitro with cytoplasmic extracts of *E. coli* (Ref. 50 and references therein). The chromosomes in some organisms may be packaged by both nucleosomes and crowding, perhaps at

the same time or at different times in the life cycle. Although we have speculated about the consequences of the crowding type of DNA packaging,⁽⁵¹⁾ crowding has not yet been demonstrated in vivo.⁽⁵²⁾

Conclusion

It is widely held that most eukaryotes differ from most eubacteria with respect to the chromosomal properties considered above. This is undoubtedly true for some of these properties. The counter-examples discussed above, however, show that there is no obligatory association between any of these chromosomal properties and the presence or absence of a nuclear membrane. Some of the apparent prokaryotic/eukaryotic chromosomal differences can be attributed to difficulties in analyzing structural features in situ with tiny bacterial nucleoids and to the arbitrary choice of representative organisms for comparison. Consequently, prokaryotic and eukaryotic organisms may not be as different as is generally believed. A dichotomy among organisms can be seen, however, when nucleosome-based packaging is considered, provided that one accepts the conclusion that the absence of nucleosomes in some dinoflagellates⁽⁵³⁾ and *N. eucaryotum*⁽⁵⁴⁾ represents loss of that character. In this dichotomy, some members of the Euryarchaeota are grouped with the eukaryotes and are distinguished from non-nucleosomal cell types, which include most prokaryotes that have been examined. The distinction also applies to the presence, in nucleosomal organisms, of histones because non-nucleosomal organisms lack these proteins.^(3,4)

The presence of histones and nucleosomal packaging of chromosomal DNA probably predates the development of a nuclear membrane, and so distinguishing chromosomes on the basis of DNA packaging is likely to reflect a deeper phylogenetic branching. This view focuses attention on delineating nucleosomal archaea from non-nucleosomal groups. Such characterization, which is just beginning, may be important for identifying the modern representatives of the archaea likely to have participated in the origin of eukaryotes. For example, Gupta⁽⁵⁵⁾ proposed that eukaryotes originated from a fusion between a member of the Crenarchaeota and a Gram-negative-like eubacterium; however, the archaea in which nucleosomes have been identified are in a different group, the Euryarchaeota. In contrast, eukaryotic origin models involving symbiosis do specify the Euryarchaeota as one of the symbionts.⁽⁵⁶⁾ Thus, chromosome structure may be a useful character for phylogenetic inference.

Finally, we note that bacterial species in the order Planctomycetales and bacteria-like sponge symbionts (some having an archaea-like cell wall appearance) contain a nucleoid bounded by a single^(57–59) or a double⁽⁶⁰⁾ bilayer membrane. It is now important to determine whether such membranes represent homologies between lower and higher

organisms or convergent evolution. In either case, it has become apparent that the principal character currently used to distinguish bacterial cells from higher life forms, the nuclear membrane, is not a very good discriminator.

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