2   Mitochondria: Key to Complexity

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2.1   Introduction

All known eukaryotic cells either have, or once had, and later lost, mitochondria (which is to say, the common ancestor of mitochondria and hydrogenosomes; Gray et al. 1999, 2001; Embley et al. 2003; Tielens et al. 2002; Boxma et al. 2005; Gray 2005). If this statement is upheld (discussed elsewhere in this volume), then possession of mitochondria could have been a sine qua non of the eukaryotic condition. That cannot be said of any other organelle. The eukaryotic cell apparently evolved only once – all modern eukaryotes are descended from a single common ancestor – and that ancestor had mitochondria (Martin 2005; Lane 2005; Martin and Müller 1998).

By definition, there are no eukaryotic cells without a nucleus, but it is a surprise that there are no eukaryotes that did not have mitochondria in their past. The implications have not yet been properly digested. Why was this? What advantage did the mitochondria offer? Whatever the advantage, it was not trivial. Bacteria and archaea ruled the Earth for three billion years (Knoll 2003). During this time, they evolved a dazzling wealth of biochemical variety, making the eukaryotes look impoverished (Martin and Russell 2003). Yet the prokaryotes failed to evolve greater morphological complexity: although some bacteria might best be thought of as multicellular organisms, their degree of organisation falls far short of eukaryotic attainments (Kroos 2005; Velicer and Yu 2003). In general, bacteria today seem to be no more complex than in the earliest known fossils (Knoll 2003; Maynard Smith and Szathmáry 1995). Such lack of 'progress' seems to be true of their biochemistry too: all the most important geochemical cycles were apparently in place by 2.7 billion years ago, implying that prokaryotes had already by then evolved oxygenic photosynthesis, sulphate reduction, fermentation, oxidative phosphorylation, methanogenesis, denitrification and nitrification (Martin et al. 2003; Lane 2002; Anbar and Knoll 2002; Nisbet and Sleep 2001; Canfield et al. 2000; Castresana and Moreira 1999; Canfield 1998).

The traditional long list of differences between prokaryotic and eukaryotic cells has been gradually eroded as exceptions are found to each. There are prokaryotes with structures resembling a nucleus, straight chromosomes, cytoskeleton, giant size, internal membranes, multiple replicons, introns, mitotic-like apparatus, gene regulation, inter-cellular signalling, genetic

recombination, and indeed with endosymbionts (Margolin 2005; Jones et al. 2001; van den Ent et al. 2001; Vellai and Vida 1998; Fonstein and Haselkorn 1995). There has been a revolution in our perception of the prokaryotic state (Gitai 2005). But if all these supposedly 'eukaryotic' traits are found in prokaryotes, why do we not see a continuum in complexity between prokaryotes and eukaryotes? Some might argue that we do, indeed that such a spectrum of eukaryotic traits in prokaryotes is already good evidence of a continuum. Even so, there is still a void – although there is some degree of overlap in cell biology, no prokaryotes ever gave rise directly (without endosymbiosis) to organisms even of the complexity of protozoa.

The distinction between prokaryotes and eukaryotes is essentially one of degree. Prokaryotes made a start with most aspects of molecular organisation, then stopped short. The eukaryotes took up the baton and ran. Pico-eukaryotes, discovered at the turn of the millennium to thrive in surprising abundance in extreme environments, such as iron-rich rivers (Amaral Zettler et al. 2002) and deep oceans (López-García et al. 2001), are similar to prokaryotes in their size and complexity. Even so, they have a nucleus, straight chromosomes, and organelles including tiny mitochondria (Baldauf 2003). In general, however, eukaryotes are 10,000–100,000 times the size of prokaryotes, and have genomes to match. Including non-coding DNA, no known prokaryote has a genome larger than about 10 Mb (megabases), whereas eukaryotes have expanded their genome size up to an extraordinary 670,000 Mb in *Amoeba dubia* (200 times larger than the human genome), a range of more than 4 orders of magnitude (Cavalier-Smith 1985, 2005; Gregory 2001; Cavalier-Smith and Beaton 1999).

This distinction is usually ascribed to nuclear factors, such as straight chromosomes, or to the invention of meiotic sex, which has the potential to postpone mutational meltdown, and so enable an expansion in genome size (Kondrashov 1988; Ridley 2001). On the other hand, if recombination can indeed prevent mutational meltdown (and there is little evidence to show it does; Keightley and Eyre-Walker 2000; Elena and Lenski 1997), then so too in principle could lateral gene transfer in bacteria. Despite the ubiquity of lateral gene transfer, indeed perhaps because of it, as I will argue, bacteria still do not expand their genome size (Konstantinidis and Tiedje 2004; Kunin and Ouzounis 2003; Gregory 2002). Similarly, if straight chromosomes were all that was needed to enable multiple origins of replication, then why could bacteria like *Borrelia burgdorferi* and several species of *Streptomyces*, which have straight chromosomes (Fonstein and Haselkorn 1995; Baril et al. 1989), not expand their own genome sizes up to eukaryotic proportions? Indeed, other bacteria, such as *Pseudomonas cepacia* and *Rhodobacter sphaeroides*, do have multiple replicons, but still do not expand their genome size (Vellai et al. 1998; Fonstein and Haselkorn 1995). Likewise, if the accumulation of DNA in eukaryotes was linked to the proliferation of selfishly replicating elements like transposable elements and retroviruses, as some have argued (Doolittle and Sapienza 1980; Orgel and Crick 1980), then why are bacteria virtually

immune to this? If the duplication of selfish DNA has the power to expand genome size, and this is an advantage (Zuckerkandl 2002), why did prokaryotes not take advantage?

2.2   Size

In short, the size, complexity and genomic expansion of eukaryotic cells cannot obviously be explained in terms of one of the two characteristic eukaryotic traits, the nucleus. Might it then be explained in terms of the other key trait, the mitochondria? Traditionally, mitochondria have been thought of as an optional extra for eukaryotic cells, an efficient power supply, but little more (Keeling 1998). Such a view is intuitive, and perhaps befits their status as an organelle, but not their newfound status as co-progenitors of the eukaryotic cell (Martin and Müller 1998; Martin 1999; Martin et al. 2001). The emasculated conception of mitochondrial significance was reinforced by Cavalier-Smith's depiction of the archezoa as primitive amitochondriate eukaryotes (Cavalier-Smith 1987, 1989), implying that mitochondria were an asset rather than a necessity. But this view is not compatible with more recent evidence that mitochondria were a necessity, a sine qua non of the eukaryotic condition; and Cavalier-Smith (2002) no longer holds that the archezoa are primitively amitochondriate. Might it be instead that mitochondria, by compartmentalising ATP generation, enabled greater cell volume; and that genomic expansion and reorganisation were a consequence of larger cell volume?

The idea that the genomic expansion of the eukaryotes was made possible by the acquisition of mitochondria has been argued by Vellai and Vida (Velai et al. 1998; Vellai and Vida 1999), and they make several important points. In particular, they argue that energetic compartmentalisation overcame the ATP subsaturation characteristic of bacteria. They imply that it did so by facilitating phagocytosis, which requires (1) a large ATP investment to change shape by altering cytoskeletal structure dynamically, (2) loss of the cell wall, permitting vigorous changes in shape and (3) specialisation of the plasma membrane for invagination into food vacuoles, which perhaps could only be achieved after it had been freed from its commitment to chemiosmotic proton pumping. According to Vellai and Vida, this ensemble of changes is only made possible by compartmentalisation of ATP production, effectively giving eukaryotic cells multibacterial power, analogous to multiple horsepower. This is almost certainly true, and might well help explain why bacteria do not practise phagocytosis.

But there are two problems with this argument as an explanation for the origin of eukaryotes, rather than the process of phagocytosis itself. First, the key assumption is that prokaryotes are typically subsaturated with ATP, whereas eukaryotic cells are somehow not – that compartmentalisation was

enough to improve the efficiency of respiration and cure the ATP subsatura-
tion. Perhaps phagocytosis did, because it enabled predation, which in turn
meant that selection for the speed of replication was no longer overwhelming:
eukaryotes could now compete by eating the competition, rather than racing
against it. However, as Vellai and Vida (1999) themselves point out, phago-
cytosis is complex, requiring a dynamic cytoskeleton, loss of the cell wall, an
endomembrane system, and the targeting of digestive enzymes to food vac-
uoles. These did not evolve overnight, and might well never have evolved at
all in fungi (Martin et al. 2003; Martin 2005), which are nonetheless morpho-
logically complex. In the meantime, we are faced with the question: how did
energy compartmentalisation, in itself, solve ATP subsaturation, to permit
genomic expansion? The problem is all the more acute, as eukaryotic cells are
larger, and presumably have a higher demand for ATP to complete cell divi-
sion. They have mitochondria, certainly, but with no better access to food
they have no advantage, just a lot of hungry mitochondrial mouths to feed.
There is no evidence that bacteria are any less saturated with ATP than are
eukaryotes. It seems that the argument from horsepower puts the cart before
the horses.

2.3   Compartments

The second problem relates to the nature of energy compartmentalisation: if
it was such a big advantage, why did bacteria not solve it for themselves?
There are good examples of energy compartmentalisation in bacteria, such as
Nitrosococcus and *Nitrosomonas* (Madigan et al. 2002), which do have a
eukaryotic 'look' about them. Furthermore, the speed and efficiency of bac-
teria at generating ATP often massively outstrips that of eukaryotic cells. For
example, the speed of ATP production by means of oxidative phosphoryla-
tion in *Azotobacter* is 5,000 times faster than in ourselves per gram weight
(Schatz 2003). Fermentation is also typically faster, giving fermenting bacte-
ria an edge in terms of replicative speed from the same resources (Pfeiffer
et al. 2001; Cox and Bonner 2001). If bacteria outstrip eukaryotes in ATP pro-
duction, they should be less subsaturated in ATP. And if they can compart-
mentalise their own ATP production, as some certainly can, then surely they
have every advantage. Why did they not use them, and expand in genome size
and complexity as the eukaryotes did?

The answer I shall put forward in this chapter lies in the need for tight
genetic control over electron transport linked to chemiosmotic proton pump-
ing, in particular to the requirement for co-localisation of genes with the res-
piratory chains to control redox poise in mitochondria, as argued by John
Allen in Chap. 3 of this volume. I shall take this hypothesis a step further, to
propose that only endosymbiosis could compartmentalise the correct core
contingent of genes in the energy-producing organelles of eukaryotes (both

mitochondria and chloroplasts) and that the ability to regulate electron transport and chemiosmotic proton pumping over a wide area of internal membranes led directly to the greater complexity of the eukaryotes. I shall make four key points:

1. Bacteria are limited in their size and genetic complexity by heavy selection for fast replication and small genome size. The selection pressure to lose genes is set against the ubiquity of gene duplication and lateral gene transfer, which mitigate the potential calamity of losing necessary genes during short periods of disuse.

2. Because chemiosmotic proton pumping takes place across the plasma membrane, a 2D sheet, ATP generation scales with the 2/3 power of cell volume. Unless bacteria alter the density of the respiratory complexes, or the speed of electron transport, their respiratory efficiency (ATP production per unit mass) tails away with increasing size. Eukaryotes escape this fate by compartmentalisation of energy generation in mitochondria, enabling larger size without a corresponding loss of energetic efficiency (assuming that cell surface area is sufficient for nutrient absorption).

3. Bacteria were unable to compartmentalise themselves in this way because a core contingent of genes is necessary to maintain redox poise. Without endosymbiosis, bacteria have no means of allocating the correct contingent of genes to regulate redox poise across large areas of bioenergetic membranes. All mitochondria have retained the same core contingent of genes (Gray 1999; Allen 1993, 2003; Allen and Raven 1996; Race et al. 1999). In this chapter, I postulate that the only way that eukaryotic cells could do so was by way of endosymbiosis.

4. Unlike bacteria, larger eukaryotic cells are energetically more efficient, according to the biological scaling laws of metabolic rate. Further, the optimal karyoplasmic ratio means that larger cells have larger nuclei. Large nuclei accumulate more DNA, as argued by Cavalier-Smith (1985, 2005), which provides raw material for greater complexity (Zuckerkandl 2002). Thus, bacteria are under a heavy selection pressure to remain small and lose DNA, while eukaryotes are under a selection pressure to become larger and to expand their genomes, giving them the raw materials necessary for greater complexity.

2.4 Dynamics of Gene Gain and Gene Loss in Bacteria

Reductive evolution is now thought to play a pervasive role in bacteria. The classic examples are obligate intracellular parasites such as *Rickettsia prowazekii* (Frank et al. 2002; Andersson and Kurland 1998), but the rule is general. Many facets of prokaryotic genome organisation attest to selection for small genomes, including the prevalence of haploid genomes, the low

proportion of non-coding DNA, the rarity of introns, the existence of a single copy most genes, and the organisation of functionally related genes into operons, lowering the costs of transcriptional regulation (Vellai and Vida 1999; Vellai et al. 1998; Fonstein and Haselkorn 1995).

The strength of the selection pressure to lose genes in free-living bacteria is hard to measure, as it can obviously be difficult to know exactly which genes are lost without trace. However, the tendency to gene loss can be estimated on the basis of the dynamic balance between gene loss and gene gain. Most prokaryotic genomes are between 0.6 and 9.5 Mb in size, a range of barely more than tenfold, despite the fact that bacteria regularly take up genes by lateral gene transfer (Cavalier-Smith 2005; Doolittle et al. 2003). In contrast, eukaryotic genome sizes vary by more than 4 orders of magnitude, from around 12 Mb in *Saccharomyces cerevisiae* up to 670,000 Mb in *A. dubia* (Cavalier-Smith 1985; Gregory 2001, 2002, 2005). Given that bacterial genomes do not expand upwards in a eukaryotic fashion, there must be a dynamic balance between gene gain and gene loss. Genes can be gained by gene genesis, in particular via duplication and divergence of genes and even whole genomes, or by lateral gene transfer from other bacteria (Kunin and Ouzounis 2003).

From an evolutionary point of view, lateral gene transfer is mainly considered in terms of its potential to confound gene trees (Doolittle et al. 2003; Feil et al. 2001). Within particular species, lateral transfer and recombination is indeed the predominant form of genetic variety, accounting for as much as 97% of variation in *Neisseria gonorrhoeae*, for example (Maynard Smith et al. 1993, 2000; Spratt et al. 2001). In this case, even the gene for 16S ribosomal RNA, often thought of as a true guide to phylogeny, part of the small, unshifting, core bacterial gene set, is in fact passed around by lateral transfer (Smith et al. 1999; Spratt et al. 2001). The scale of lateral gene transfer in other bacterial species is illustrated by two different strains of *Escherichia coli*, which differ more radically in their gene content (a third of their genomes, or nearly 2,000 different genes) than all mammals, perhaps even all vertebrates (Doolittle et al. 2003).

The extent to which lateral transfer confounds evolutionary trees is uncertain. In one study, Kunin and Ouzounis (2003) analysed the distribution of 12,762 protein families on a phylogenetic tree derived from the entire genomes of 41 species of bacteria and ten species of archaea. They examined the presence or absence of genes on this tree. If a gene was consistently present in a clade, they concluded the corresponding gene had been present in the ancestor of that clade; occasional absence was ascribed to gene loss, while fragmented distribution across distantly related species was ascribed to lateral gene transfer. The authors concluded that gene loss was the dominant driving force in prokaryotic evolution, while gene duplication was twice as common as lateral transfer. The number of protein families involved in lateral gene transfer was estimated to be 25–39% of the protein families examined, while 60% of families appeared to have been inherited vertically.

This is good news from the point of view of the veracity of gene trees – they are not completely garbled by lateral gene transfer across species and clades, and vertical inheritance apparently still shines through – but the study still corroborated the importance of lateral gene transfer as one of the major driving forces of prokaryotic evolution, partially balancing the strong tendency to gene loss. In fact, the importance of lateral transfer is likely to be greater than suggested by this study, as the estimates of its prevalence were insensitive to transfers within single species or closely related species, which are presumably more common than lateral transfer across clades (because the genes are closely related, so homologous recombination is likely to be successful). Despite these shortcomings, this large study nonetheless illustrates well the dynamic balance between gene loss and gene gain in bacteria.

The benefits of a dynamic balance can best be understood in the context of a heavy pressure to lose genes, combined with fluctuating environmental conditions. The pressure to lose genes derives from the time and energy cost of replicating large genomes. Under optimal conditions, cell division in *E. coli* takes just 20 min, even though it takes 40 min to replicate the full genome (O'Farrell 1992). The only way *E. coli* can complete its cycle of cell division in 20 min is by initiating the second round of genome replication before the first round is complete (O'Farrell 1992; Donachie and Blakely 2003). Clearly, the smaller the genome, the less time it takes to replicate. This applies to cells growing in a single population, rather than to different species, which replicate at disparate speeds that are not necessarily related to their genome size (Vellai, personal communication). In a striking study, Vellai et al. demonstrated the importance of genome size in *E. coli* by engineering three plasmid vectors, each of different size (6, 11.8 and 15.5 kb), but all of them encoding a gene conferring resistance to the antibiotic ampicillin. Cells containing vector DNA were cultured in the presence or absence of ampicillin, and growth curves were constructed. In the absence of ampicillin, all three cultures grew at a similar rate, but under the restrictive phase (when the culture medium begins to be exhausted), the proportion of viable cells retaining their vector DNA declined in proportion to the size of the vector. In other words, in the restrictive phase, equivalent to natural conditions, cells were under a heavy selection pressure to jettison unnecessary DNA. When cells were grown in the presence of ampicillin, the outcome was very different. Cells losing their vector were rendered vulnerable to ampicillin, so all viable cells had retained the vector. Now, in the restrictive phase, the cells with the smallest plasmids (6 kb) outgrew the cells with the largest plasmids (15.5 kb) by an order of magnitude over a 12-h period (10^{10} versus 10^9 cells). So, for *E. coli*, larger genomes are penalised by natural selection, especially under restricted conditions, when cells are subsaturated with ATP.

These studies are revealing, for they imply that there is a strong pressure to lose superfluous genes from a bacterial population, a pressure which is presumably balanced by the ability to regain genes by lateral gene transfer, either from within the species or elsewhere, whenever conditions change

again. Plasmid transfer could easily balance gene loss from vectors – this is well known to account for the swift rise of antibiotic-resistant strains among pathogens (Maynard Smith et al. 2000; Spratt et al. 2001) – but presumably the same process also occurs in bacterial chromosomes, if more slowly, given a dynamic balance between gene loss and gene gain, and the roughly constant size of bacterial genomes. Gene loss is random, and may cause cell death (if an essential gene is deleted), or decrease growth rates (if functioning but non-essential genes are deleted); any clones that are affected in this way will be eliminated by natural selection. On the other hand, DNA loss can be advantageous if it eliminates unused sequences, for this speeds replication, if only slightly, as shown by Vellai et al. (1998). Importantly, it takes a prolonged period, relative to environmental fluctuations (such as seasonal fluctuations in substrate concentration) to lose all copies of any particular gene from a population or species, in any one environment. Furthermore, bacteria can recombine gene fragments to regenerate a functional gene by homologous recombination (Maynard Smith et al. 1993; Vellai, personal communication). Indeed, DNA sequencing has revealed that individual bacterial genes have a mosaic structure that could only have arisen by homologous recombination, even in bacteria not previously thought to show natural transformation (Maynard Smith et al. 2000; Spratt et al. 2001). It is therefore likely that, if conditions fluctuate, then functional copies of deleted genes can be regenerated within a population as a whole. Despite the heavy rates of gene loss over evolutionary time, it must be relatively rare, in terms of the dynamics of population turnover, to lose a gene from a population altogether. As Vellai (personal communication) put it, "Natural bacterial populations contain dispersed genomes, which reflects a community-like organization of the genetic material."

Dispersed genomes are the optimal stable strategy for fluctuating environmental conditions, as only cells that can *draw on* large genomes can survive most exigencies, while at the same time bacteria that invariably *retain* large genomes are outcompeted by bacteria with smaller genomes, which are viable *at that particular time*. The combination of gene loss with lateral transfer enables speedy replication and genetic resourcefulness: bacteria that economise and load genes dynamically thrive in place of bacteria with large, metabolically flexible genomes, or bacteria with small but inflexible genomes. If conditions are relatively stable, however, the rules shift. If lean resources are the rule, bacteria can tolerate larger genomes, as the metabolic flexibility can enable ATP to be produced more effectively than competitors. This tendency is borne out by an examination of 115 fully sequenced bacterial genomes by Konstantinidis and Tiedje (2004). They found that the bacteria that have the largest genomes dominate in environments where resources are scarce but diverse, and where there is little penalty for slow growth, such as soil. Soil bacteria produce on average three generations in a year, so there is less selection for speed than for any replication whatsoever (Konstantinidis and Tiedje 2004). Under such conditions an ability to take advantage of scant

resources is important, which requires more genes to encode the metabolic flexibility needed (Stêpkowski and Legocki 2001). It is no accident that *Streptomyces avermitilis*, ubiquitous in the soil, is metabolically versatile with a big genome to match. Even so, there is still selection for small size in relation to other versatile bacteria, and presumably this sets the bacterial genome ceiling of about 9.5 Mb. In other words, even the most versatile bacteria have small genomes compared with those of the eukaryotic cells living in the same environment. So how were the eukaryotes released from a selection pressure that stifles even the most versatile bacteria?

2.5   ATP Regulation of Bacterial Replication

For both bacteria and eukaryotes, ATP subsaturation is the norm. On a global scale it is clear that this is the case. In optimal conditions, *E. coli* can divide every 20 min, or 72 times in a day. A single *E. coli* bacterium weighs about 10^{-12} g, so 72 cell divisions in a day corresponds to an amplification of 2^{72} $(= 10^{72} \times \log 2 = 10^{21.6})$, or an increase in weight from 10^{-12} g to 4,000 t (O'Farrell 1992). In 2 days the mass of exponentially doubling *E. coli* would be 2,664 times larger than the mass of the Earth (which weighs 5.977×10^{21} t). The fact that this does not happen shows that nutrient supply is an overriding limiting factor.

The speed of bacterial replication is closely tied to nutrient availability. DNA replication is controlled directly by ATP availability, through the formation of orisomes (Cunningham and Berger 2005; Leonard and Grimwade 2005). These create a small bubble of unwound DNA within the replication origin, which recruits the helicase. A critical first step is the stable unwinding of the chromosomal replication origin, *oriC*, by the multiprotein orisome complexes, comprising the initiator DnaA and modulator proteins that bend DNA (Leonard and Grimwade 2005). About 20 DnaA molecules bind to at least five 'DnaA boxes' in the *oriC* region of the chromosome (McGarry et al. 2004; Messer 2002). To bind to the DnaA boxes, the DnaA proteins must also bind to ATP or ADP (Messer 2002). The DnaA protein binds either nucleotide with equal avidity, but it seems that only the ATP-DnaA is active in initiating replication, at least in critical boxes (McGarry et al. 2004). ATP binding promotes an allosteric modification, and does not provide energy for the unwinding reaction, as non-hydrolysable ATP analogues are equally effective (Leonard and Grimwade 2005).

Presumably, such a mechanism calibrates the balance of ATP to ADP within the cell. During active replication, some 1,000–2,000 DnaA proteins are present in the cell, and occasionally as many as 10,000 (Donachie and Blakely 2003). These bind to either ATP or ADP, which in turn enables thermodynamic control over bacterial replication. This is because the Gibbs free energy of ATP hydrolysis depends on the ratio of ATP to ADP, not their total

concentration (Jensen et al. 1995). The energy of ATP resides not in a 'high-energy' bond, as is often stated (the bond is actually quite low in energy, which is why it is so easy to break, and thus useful), but in the displacement of the chemical equilibrium towards ATP from ADP (Harold 1986, 2001). Increasing the ATP concentration is only helpful if it is at the expense of ADP concentration. The cell must have a mechanism to determine whether ATP supplies are sufficient at the outset to complete cell division, and this depends on the thermodynamic driving force – the ratio of ATP to ADP. The cell can monitor the driving force simply by means of a molecular switch that has an equal binding affinity for ATP or ADP (reflecting their concentration ratio), but which is unequally activated by nucleotide binding. To be genuinely sensitive to the ratio also requires a large statistical 'sample size' (just as political polls are only reliable if they question a representative section of the population) and this may account for the surprisingly high number of DnaA proteins, totalling several thousand.

Such thermodynamic control of cell replication is also evident in DNA supercoiling (Jensen et al. 1995; Koedoed et al. 2002; Snoep et al. 2002). Negative supercoiling facilitates the opening of the double helix, which is necessary for growth-related processes such as transcription and replication. This is achieved with the aid of enzymes like DNA gyrase. In vitro studies with isolated DNA gyrase from *E. coli* show that the activity of the enzyme is sensitive to the ratio of ATP and ADP, whereas the absolute concentrations of the nucleotides have no effect on the enzyme activity (Westerhoff et al. 1988; Westerhoff and van Workum 1990). When cells run out of growth substrates (or are in the presence of respiratory uncouplers), the changes in DNA supercoiling stimulate expression of some genes, while repressing expression of others, thereby adjusting the pattern of gene expression to the new conditions (Jensen et al. 1995). In vivo, in *E. coli* at least, when the ratio of ATP to ADP falls to less than 1, there is a general inhibition of transcription and replication, and so of cell division (Jensen et al. 2001). Thus, it seems there are a number of ways in which cells can use the thermodynamic indicator of the ATP to ADP ratio to control cell division.

Another control over bacterial division is cell size, which is also regulated by the ATP to ADP ratio (Donachie and Blakely 2003). Initiation of replication takes place at a fixed cell volume, the 'initiation volume', which is constant under a wide variety of growth conditions, despite the fact that cell size at division varies greatly at different growth rates (Donachie 1968). Once DNA replication has been initiated, the cell then enters an 'eclipse period', during which time it cannot start a second cycle of DNA replication. The end of the eclipse period is related to cell volume, by way of a mechanism that involves the binding of DnaA proteins to various DnaA-binding sequences in DNA, including the *oriC* boxes mentioned before, as well as a number of other sites, such the as *datA* locus (Donachie and Blakely 2003; Christensen et al. 1999). The latter may bind as many as 370 DnaA proteins (Kitagawa et al. 1996; Ogawa et al. 2002). During the eclipse period, most of the DnaA

proteins are bound to ADP rather than to ATP, though exactly how the shift is brought about is unknown (Donachie and Blakely 2003). One plausible factor is that the DnaA protein has a weak ATPase activity, which is needed for it to dissociate from DNA: the DnaA protein hydrolyses the bound ATP, leaving it free in the inactive ADP-bound form (Messer 2002). However, such a mechanism could only account for a rather small proportion of perhaps 2,000 DnaA proteins, so there is more to it than that (Donachie and Blakely 2003). Regardless of the precise mechanism, the eclipse period is linked to the ATP to ADP ratio. It begins shortly after initiation of DNA replication and persists for about a third of the cell cycle, during which time the DnaA-ATP to DnaA-ADP ratio reaches a minimum (Donachie and Blakely 2003). Thereafter, the ratio climbs again with de novo DnaA-ATP production, at a rate that parallels protein synthesis. This implies that the ratio of DnaA-ATP to DnaA-ADP increases in parallel with cell size, until it reaches a critical value, at which the active form can compete successfully with the inactive form for binding at *oriC*, and thus initiate a new round of DNA replication (Donachie and Blakely 2003). Such an interpretation is corroborated by mutants lacking the *datA* locus, which show asynchronous initiation of replication (Ogawa et al. 2002), and by mutants with additional *datA* copies, which have a higher initiation volume (a larger cell size at the initiation of DNA replication), consistent with delayed initiation of replication (Kitagawa et al. 1996).

All these various mechanisms tie bacterial replication to ATP production. Clearly, the more efficiently and quickly a cell produces ATP, the more quickly it can replicate. The balance between speed and efficiency is an interesting one, for fermentation is faster than oxidative phosphorylation, but has a substantially lower yield of ATP, for it only partially oxidises the substrate. This leaves substrate for cells capable of oxidative phosphorylation. Thus, cells growing by fermentation typically outcompete respirers, at least in the short term (Pfeiffer et al. 2001; Cox and Bonner 2001) Afterwards, their growth is rapidly curtailed by the exhaustion of substrate. If anaerobic bacteria excrete waste products such as ethanol into their surroundings, then slowly replicating aerobic bacteria may ultimately outgrow them by oxidising the ethanol. The most able replicators might therefore be bacteria capable of facultative anaerobic respiration, exactly those proposed by Martin and Müller (1998) in the hydrogen hypothesis to be the free-living ancestors of the mitochondria, such as *Rhodobacter* (see also Müller and Martin 1999; Tielens et al. 2002). Interestingly, an analysis by Pfeiffer et al. (2001) suggests that obligate aerobic cells, which have a high yield but low rate of ATP production, might do best when grouped in clusters, hiving off substrate for later use. Such collaborative behaviour could have been an incentive towards the origin of multicellular colonies in eukaryotes (Pfeiffer et al. 2001; Cox et al. 2001). In prokaryotes, however, obligate aerobic respiration is most commonly found in obligate intracellular parasites such as *Rickettsia* (which may gain by maximising ATP yield in their constricted intracellular environment)

and some soil bacteria, where growth is slow, owing to scarcity of nutrients, so ATP yield is critical (Stêpkowski and Legocki 2001).

In considering the efficiency and speed of ATP production in bacteria, size matters. Bacteria respire over their plasma membrane, pumping protons into the periplasm (in Gram-negative bacteria, or the equivalent space between the plasma membrane and the cell wall in the other prokaryotes; Vellai et al. 1999; Harold 2001; Lane 2005). This means that the efficiency of ATP production depends on the surface-area to volume ratio. As bacteria become larger, their chemiosmotic ATP production depends on the surface area of the plasma membrane, which increases with the square of cell dimensions, whereas the volume of the cell increases with the cube of its dimensions. Overall, as cell size increases, the metabolic rate scales with the 2/3 power of cell volume, all other factors being equal. Excluding vacuoles (which, for example, explain the giant size of *Thiomargarita namibiensis*, with its diameter of up to 300 μm; Schulz et al. 1999), larger bacteria are composed of more structural macromolecules. These in turn consume more ATP for their synthesis, so larger bacterial cells must have a higher requirement for ATP, coupled with a relatively lower capacity for producing that ATP. Presumably, the ATP to ADP ratio takes longer to arrive at the critical threshold, such that the eclipse period is longer and cell replication is accordingly slower. As bacteria become larger, their energetic efficiency tails away, a serious cost liable to be penalised by selection.

There are of course many adaptations that bacteria could make, which influence the speed or efficiency of ATP production. Fermentation, for example, does not depend on the surface area and so is not affected by size. Relatively simple changes in cell shape affect the surface-area to volume ratio: rods have a larger surface area relative to their volume than spheres, perhaps conferring a slight selective advantage on bacilli compared with cocci. The kinetic efficiency of respiratory enzymes could be optimised, as can their packing density within the membrane. All these changes have costs attached, however. Fermentation has a low ATP yield, which probably restricts cell size, as smaller cells can replicate more times given limited substrate; complex morphology requires more genes to encode it, and macromolecules to build it; optimising the efficiency of respiratory enzymes requires greater selectivity for their substrates or electron acceptors, to the exclusion of others that may at times be more abundant. Increasing packing density of respiratory complexes must affect the density of other membrane proteins needed for nutrient absorption, homeostasis, motility, or sensing the concentration gradient of various substrates or toxins. And if the packing density of all membrane proteins rises, then the fluid properties of the lipid phase must change, again with potentially detrimental consequences. While species adapted to different environments might benefit from any of these adaptations, within a single population of cells competing for the same resources, the most important factor is cell size. If all else is roughly equal, large cells are penalised by natural selection, as they produce ATP less efficiently than small cells, and so

are slower to replicate. As a result, prokaryotes are under a heavy selection pressure to remain small in size, just as they are under a heavy pressure for small genomes (Lane 2005).

2.6   Redox Poise Across Bioenergetic Membranes

There is one change that bacteria could make to enable larger size without a loss of energetic efficiency, and that is internalising the bioenergetic membranes. If the speed of respiration in the cell depends on the surface area of bioenergetic membranes, then obviously the more membranes the faster the ATP production. What is more, internalising respiration frees up the plasma membrane to specialise in other tasks, such as the absorption of nutrients, tasks that no longer need to compete for space with the respiratory or ATPase complexes. This is of course exactly what the eukaryotes did, for the surface area of bioenergetic membranes in eukaryotic cells depends on the density of cristae and the total number of mitochondria, but is no longer tied to the external surface area of the cell. The question is: why did bacteria not internalise their own bioenergetic membranes? In some cases, such as *Nitrosomonas* and *Nitrosococcus*, they did (Madigan et al. 2002), so the question is again really one of degree: why did the bacteria once again make a start, and then stop short?

The answer is to be found not in the bacteria themselves, but in the mitochondria. The mitochondria were, after all, once bacteria, and their respiratory prowess is bacterial (Berry 2003). Within the context of the cell as a whole, what are the differences between the internal membranes of bacteria like *Nitrosocossus* and the cristae of mitochondria? The answer, surely, is *genes*. All known mitochondria in all known eukaryotes have retained a small genome, closely associated with the inner mitochondrial membrane (Gray et al. 1999, Burger et al. 2003). In contrast, the internal membranes of *Nitrosococcus* are not associated with any core contingent of genes (Madigan et al. 2002). Why mitochondria retained their own genes is not generally agreed, but Allen (1993, 2003) has put forward a convincing case that both chloroplasts and mitochondria retain their genomes to control redox poise during electron transport coupled to chemiosmotic proton pumping. If so, then perhaps bacteria cannot expand their internal membranes because they cannot localise the correct contingent of genes with their bioenergetic membranes, except by way of endosymbiosis.

Allen's theory of redox poise, and the evidence supporting it, are discussed Chap. 3 in this volume. Here, I want to make a few general observations on necessity and workability. Each mitochondrion needs a genome because the speed of electron flow down the respiratory chains depends not just on supply and demand (concentration of NADH, O_2, ADP and inorganic phosphate) but also on the number and redox state of respiratory complexes (Allen 1993,

2003; Allen and Raven 1996; Race et al. 1999; Otten et al. 1999; Blackstone 2000). If there are too few complexes, electrons pass slowly down the respiratory chains from NADH to oxygen, even if demand is high and substrates and electron acceptors are abundant. Conversely, if there are too many complexes, electrons accumulate in the chain, perhaps resulting in a higher free-radical leak (Race et al. 1999; Allen 1993, 2003; Lane 2005). Thus, it is necessary to regulate the turnover of respiratory complexes within individual mitochondria to correspond with demand, maintaining redox poise and minimising free-radical leak. Respiration is fastest when a balanced number of electron carriers are balanced in their redox state, which is to say 50% are reduced and 50% oxidised (redox poise; Allen 2003).

The difficulty is a matter of targeting. If one mitochondrion needs more cytochrome oxidase, it could signal its deficiency to the nucleus, stimulating transcription of nuclear genes; however, if there are several hundred mitochondria in a cell, then there is a problem targeting de novo cytochrome oxidase to the correct mitochondrion. If the new protein is delivered to all the mitochondria, 99% will receive too much, and 1% will receive too little. Redox poise is lost in all the mitochondria. In contrast, if a core of genes is retained in individual mitochondria, then the signal is sent only as far as the local genome. It responds by synthesising more cytochrome oxidase on site, and restores the correct balance. Such a rapid local response could take place in any of the cell's mitochondria and might in principle operate quite differently in different mitochondria in the same cell at the same time. Redox poise is retained in all mitochondria. In other words, by having a small genome within each mitochondrion, despite the costs (such as maintenance costs of numerous copies of the requisite genetic machinery, and a fast mutation rate of mitochondrial DNA), the cell as a whole can maintain redox poise across a wide area of bioenergetic membranes (Allen and Raven 1996).

Exactly how such signalling works is not known. In fact, however, a simple mechanism exists that depends on no more than the biophysics of electron flow down the respiratory chains, as argued by Blackstone (Blackstone and Green 1999; Blackstone 2000). In essence, the rate of free-radical leak does not depend on the speed of respiration, but rather on the redox state of the respiratory complexes: a high reduction state of complex I (above about 90% reduced) leads to a high free-radical leak (Turrens 2003; Barja 1998; Brand et al. 2004; Skulachev 1998, 2004). This means that the signal might simply be free-radical leak: a rise in free-radical leak alters gene activity through the action of redox-sensitive transcription factors, or differential stability of transcripts (of which there are many examples; Allen 2003). So, for example, if cytochrome oxidase (complex IV) is deficient, the redox state of complex I will rise, and with it free-radical leak. One question is how the cell interprets this signal to 'know' that more cytochrome oxidase is needed. Free-radical leak also rises if there is a low demand for ATP: the redox state of complex I then rises as electrons accumulate in the chain, but this leak could not be

relieved by building new complexes. In principle, though, the mitochondria could detect ATP levels and so combine two signals: 'high ATP' and 'high free radicals'. An appropriate response would now be to dissipate the proton gradient, to maintain electron flow, and there is indeed evidence that this happens (Brand et al. 2004). In contrast, if there were not enough respiratory complexes, ATP levels would decline and electrons would again accumulate in the respiratory chains. Now the signal would combine 'low ATP' with 'high free-radical leak'. This system could in theory discriminate the need for more respiratory complexes from low ATP demand. Similar signalling systems operate at a cellular level to signal apoptosis in eukaryotes ('high free radicals', 'low ATP'; Zamzami et al. 1995, Richter et al. 1996; Ott et al. 2002) and homologous recombination in simple eukaryotes like *Volvox carteri* and *S. cerevisiae* ('high free radicals', 'high ATP'; Brennan and Schiestl 1998; Nedelcu et al. 2004). In the case of *V. carteri*, a twofold rise in free-radical production activates the sex genes, leading to the formation of new gametes (Nedelcu et al. 2004). Importantly, this effect can be induced by inhibitors of the respiratory chain, which increase the rate of free-radical leak (Nedelcu et al. 2004).

A problem with this model in mitochondria is that the respiratory complexes are constructed from a large number of subunits, as many as 48 separate proteins in complex I. Mitochondrial genes encode a handful of these subunits (seven in the case of complex I), but nuclear genes encode the great majority. How, then, could the mitochondrial genes dominate? They could do so if the respiratory complexes assemble themselves around a few core subunits; and there is evidence that this happens (Ugalde et al. 2004; Vogel et al. 2004; Bai et al. 2004; Cardol et al. 2002). If the mitochondrial genes encoded these critical or rate-limiting subunits, then they would control the assembly of new complexes. Effectively, mitochondria would make a construction decision, and plant a flag in the membrane; and the nuclear components then assemble around the flag. Again, there is evidence that this is indeed what happens (Chomyn 2001). Given that the nucleus serves hundreds of mitochondria at once, the total number of flags in the cell at any one time might remain fairly constant. There would be no need to change the overall rate of nuclear transcription to compensate for fluctuations in individual mitochondria, but the effect would be to keep a tight grip on the rate of respiration in all the mitochondria in a cell at once. This is indeed a plausible mechanism, for in both the mitochondria and the chloroplasts, the organellar genome encodes the core subunits of complexes (Chomyn 2001; Race et al. 1999; Allen 2003).

If these arguments are correct, then they can explain why bacteria are limited in their internal membrane systems: they are unable to retain redox control over a larger area of membranes because they cannot localise the correct core contingent of genes. In the light of the first two sections of this chapter, it is salutary to consider the penalties for redox control. The simplest way would be to copy a subset of genes and delegate it to regulate the

extra membranes – but how are the necessary genes chosen? There is no way that I can think of that does not involve foresight, and evolution has none. The only way that such delegation could work would be to replicate the entire genome, and whittle away at one of the two genomes until all superfluous genes were deleted or transferred to the host cell (as happened in mitochondria). But which genome should lose its genes? Both must be active for redox control to work. In the meantime, however, the bacterium has two active genomes, each under a heavy selection pressure to lose superfluous genes. Either genome might be expected to lose some genes, but two competing genomes in the same cell raises the spectre of genomic conflict, certainly not aiding it in competition with other cells (Cosmides and Tooby 1981; Hurst and Hamilton 1992; Partridge and Hurst 1998; Ridley 2001).

Genomic conflict might be prevented if it were possible to demark the sphere of influence of each genome. The eukaryotes solved the problem by sealing off the mitochondrial genome in a double membrane. This is not possible in bacteria, however. If the spare set of genes were sealed off, there would be no way of extracting ATP. The ADP/ATP carriers do not exist in bacteria, with the exception of obligate intracellular parasites such as *Rickettsia*, whose ADP/ATP carriers bear some similarities to plastid carriers and appear to branch deeply in the eukaryotic tree (Amiri et al. 2003; Andersson et al. 2003); but exporting ATP is of course a suicidal trait for free-living bacteria. The mitochondria ADP/ATP carriers, along with the family of 150 mitochondrial transport proteins to which they belong, are purely a eukaryotic invention: their gene sequences are closely related in all eukaryotes, but there are no similar bacterial genes (Löytynoja and Milinkovitch 2001; Santamaria et al. 2004). This implies that the ADP/ATP carriers had evolved in the last common ancestor of all the eukaryotes, before the divergence of the major groups.

The eukaryotes had time to evolve ADP/ATP carriers because the metabolic syntrophy between the two partners of the original chimeric eukaryote was stable, providing ample time and stability for evolutionary changes to take place (Martin and Müller 1998). In the case of bacteria evolving by natural selection (rather than symbiosis), however, there is no corresponding stability. Simply duplicating a gene set and sealing it off in a membrane could in itself provide no advantages in the interim. Far from it: maintaining extra genes and membranes without any benefit is costly, and would be eliminated by natural selection. Whichever way we look at it, selection pressure is likely to jettison the burdensome additional genes needed for respiratory control over a wide area of membranes in bacteria. The most stable state is always a small cell that respires across the plasma membrane, using a periplasmic space to generate a chemiosmotic proton gradient – in short, a bacterium. Because selection is probabilistic, small cells will almost invariably be favoured over larger, inefficient, free-radical-leaking competitors. And that is why bacteria are still bacteria.

2.7   Allometric Scaling of Metabolic Rate and Complexity

Metabolic syntrophy, such as posited by the hydrogen hypothesis, explains why only endosymbiosis is stable enough over evolutionary time to enable multiple core contingents of genes controlling redox poise to be co-localised with a wide area of bioenergetic membranes. Although such endosymbiosis is common in eukaryotes, it is far less common in bacterial communities, which tend to live together but not inside one another. A major obstacle to endosymbiosis in bacteria is the absence of phagocytosis among prokaryotes, and indeed Cavalier-Smith (2002) has argued that the first eukaryote was necessarily phagocytic. There are nonetheless examples of bacteria living inside other bacteria, without a hint of phagocytosis, so clearly it is possible, if uncommon, as postulated by the hydrogen hypothesis (von Dohlen et al. 2001; Hoffmeister and Martin 2003; see also Chap. 4 by Sapp in this volume). If the ideas in this chapter are correct, then the advantages of eukaryotic energy generation in mitochondria can only be gained by endosymbiosis in bacteria. Because only a handful of scenarios explain how an endosymbiosis could have led to the rise of a eukaryotic cell, this helps to explain why the eukaryotes only arose once in the history of life on Earth.

There is still a question about the rise of complexity to answer, however, and it is this. Once a small, if incipiently complex, eukaryotic cell was established, what 'drove' the eukaryotes on to greater size and complexity? Gould (1997) and others have argued that there is no inherent direction in evolution, that the rise of complexity was little more than a random walk stopping off at vacant niches on route. Because the bacteria and archaea already occupied all the simplest niches, the eukaryotes had no option but to evolve in the direction of greater complexity. Nonetheless, bacteria and archaea dominated the Earth for three billion years, yet never showed any tendency towards greater morphological complexity in that time, nor indeed towards greater biochemical complexity, if the major geochemical cycles were all in place 2.7 billion years ago (Martin et al. 2003; Lane 2002; Anbar and Knoll 2002; Nisbet and Sleep 2001; Canfield et al. 2000; Castresana and Moreira 1999; Canfield 1998). Yet once the eukaryotes had evolved, there was a gearshift. In less than half the time open to bacteria, the eukaryotes developed elaborate endomembrane systems, complex cell cycles, specialised organelles, mitosis, meiosis, huge genomes, phagocytosis, predatory activity, multicellularity, differentiation, large size, and spectacular feats of mechanical engineering: flight, sight, hearing, echolocation, brains, sentience. Insofar as this progression happened over time, it can reasonably be plotted as a ramp of ascending complexity. So we are faced with the prokaryotes, with nearly unlimited biochemical diversity but no drive towards complexity, and eukaryotes, which have little biochemical diversity, but a marvellous flowering in the realm of bodily design. Is there anything about the evolution of

mitochondria that made the evolution of larger size and greater complexity not just possible, but probable?

The answer is yes: the scaling of metabolic rate with size (Kleiber 1961). Allometric scaling laws in biology have undergone a renaissance in interest since West et al. (1997) proposed a fractal model to explain the 'three quarters law' of metabolism, the apparent scaling of metabolic rate with mass to the power of 3/4 across an extraordinary 27 orders of magnitude, from respiratory complexes within mitochondria to the blue whale. In essence, the fractal model argues that metabolic rate is determined by the fractal properties of the supply network, i.e. fractal geometry constrains energy metabolism (West et al. 1997, 1999, 2002; Weibel 2002). This theory has been criticised from various points of view (Lane 2005), notably (1) three-quarter scaling is an illusion; in fact metabolic rate more commonly scales with $m^{2/3}$, where m is mass (Heusner 1991; Dodds et al. 2001; White and Seymour 2003); (2) the theory predicts that the fractal supply network constrains the resting metabolic rate, whereas in fact it can only constrain maximal metabolic rate, which scales with 0.88, not 0.75 (Bishop 1999); (3) in mammals, the scaling of the supply network generally reflects tissue demand, so capillary density scales with m in muscle tissue; (4) different tissues scale differently with body mass (Bennett 1988; Rolfe and Brown 1997; Porter 2001). Bone, for example, is metabolically inert. Because bone strength depends on cross-sectional area, and the weight it bears depends on body mass, a greater proportion of body mass must be composed of metabolically inert bone with increasing size; and this must affect the scaling of metabolic rate with mass. This last point was the basis of the allometric cascade model of Hochachka et al. (Hochachka et al. 2003; Darveau et al. 2002) using metabolic control analysis . Their model demonstrated that different tissues scale differently with body mass, according to demand and the economies of scale. They showed that, in animals, larger size is metabolically more efficient, i.e. the specific metabolic rate falls with size as tissue demand falls, and the aerobic capacity correspondingly increases. As a result, both organ size and mitochondrial density in organs fall with rising body size. Importantly, this is not a constraint of greater size, but an opportunity, which may, for example, have given rise to endothermy in mammals and birds (Lane 2005).

There has been far less work at the level of individual cells, but the scaling of metabolic rate with cell size has been studied in various unicellular organisms. Despite claims that metabolic rate scales with $m^{0.75}$ in single cells, a re-evaluation by Prothero (1986) and other groups declared the relation to be "generally not at all persuasive" (Dodds et al. 2001). Metabolic rate actually scales with an exponent of between 0.3 and 1, depending on which groups are considered (Prothero 1986). The fact that the exponent is generally less than 1 means that metabolic rate falls relative to mass as cell size increases (a cell of twice the volume has a metabolic rate that is less than double); but it is unclear whether that is an opportunity or constraint of size. In bacteria, metabolic rate often scales with $m^{2/3}$ because bacteria respire over the plasma

membrane. This is a constraint of size, and helps to explain why bacteria remain small (Vellai et al. 1998; Vellai and Vida 1999; Lane 2005). However, in animals, Hochachka et al. (2003) showed that the metabolic rate falls with size as a result of the economies of scale: an opportunity of size, not a constraint. So which of these two possibilities applies to unicellular eukaryotes? The answer is not known, but I suspect that even unicellular eukaryotes do gain from the economy of scale. As in society, the benefits depend on set-up costs, operation costs and distribution costs, and these impose upper limits on the economies of scale. But within these limits, the benefits ought to apply widely. This is because living organisms are highly conservative in their operational principles. In particular, their organisation is invariably modular. Both unicellular and multicellular organisms are composed of a mosaic of modular units. Modular functions in unicellular eukaryotes include transcription and translation, protein synthesis, glycosylation, ionic homeostasis, ribosomal assembly, membrane synthesis, vacuolar or lysosomal digestion, signalling, chemotaxis, ATP generation, motility, molecular trafficking, and so on. I imagine the economies of scale apply as much to these modular aspects of single cells as to multicellular organisms. Within the limits set by the supply network, single-celled eukaryotes become more efficient as they grow larger: the set-up costs for manufacturing 100 ribosomes are higher than for manufacturing 1,000, so fewer mitochondria are needed to provide the ATP necessary.

This idea brings us back to the question of genome size that I touched on at the start of the chapter. Large cells are energetically more efficient, but large cells usually have a larger nucleus (Cavalier-Smith 1985, 2005; Gregory 2001, 2002, 2005). Balanced growth during the cell cycle apparently requires that the ratio of nuclear volume to cell volume (the karyoplasmic ratio) is roughly constant, for reasons that are disputed (discussed in detail by Cavalier-Smith 2005). Essentially, over evolution, the nuclear size and DNA content adjusts to changes in cell volume and metabolic rate (Koz[3]owski et al. 2003; Lane 2005). As cells grow larger, they adjust by developing a larger nucleus, with more DNA, even if this extra DNA does not necessarily code for more genes. This explains the C-value paradox, and is why cells like *A. dubia* have 200 times more DNA than a human being, albeit coding for fewer genes. The extra DNA may be partly structural, as argued by Cavalier-Smith (1985, 2005), but it can also be called upon to serve useful purposes, from forming the structural scaffolding of chromosomes, to providing binding sites for transcriptional regulation (Zuckerkandl 2002; Mattick and Makunin 2005; Mattick 2001). It also forms the raw material for new genes and regulatory RNAs, building the foundations of complexity (Zuckerkandl 2002; Mattick and Makunin 2005; Mattick 2001). The sequences of many genes betray their ancestry as selfishly replicating elements (Zuckerkandl 2002). Thus, as soon as eukaryotic cells became powered with mitochondria, there was a selective advantage to them being bigger. Bigger cells need more DNA, and with that they had the raw material needed for greater complexity.

This reverses the selection pressures on bacteria: whereas bacteria are oppressed by heavy selection pressure to lose genes, eukaryotes are under pressure to gain them.

2.8   Conclusions

Bacteria are under heavy selection pressure to lose any unnecessary genes, regardless of whether they may prove necessary again in the future (Vellai and Vida 1999; Kunin and Ouzounis 2003). This pressure is balanced by the capacity to regain functional copies of deleted genes by lateral transfer from within the same population, or other species, clades, or even domains (Kunin and Ouzounis 2003). Natural bacterial populations therefore effectively have dispersed genomes, combining fast replication with the genetic versatility necessary for adapting to fluctuating conditions (Vellai, personal communication).

The speed of replication is tied to cell size and ATP availability by the thermodynamic driving force of ATP to ADP ratio (Donachie and Blakely 2003). Both the rate and the yield of ATP generation are important, favouring facultative anaerobic respiration in bacteria (Pfeiffer et al. 2001; Cox et al. 2001). Size matters because the rate of ATP generation by way of a proton gradient across the plasma membrane means that metabolic rate scales with cell mass to the power of 2/3. All else being equal, respiratory efficiency (ATP production per unit mass) declines with rising cell volume. Respiratory efficiency can be maintained in larger cells by internalising the bioenergetic membranes, and some bacteria do have specialised bioenergetic membranes enclosing an extensively folded periplasmic space (Madigan et al. 2002); however, the surface area of such membranes falls orders of magnitude short of the cristae area of energetic eukaryotic cells (Lane 2005).

The surface area of internal membrane systems in bacteria is restricted by the need to maintain tight redox poise during electron transport. This is achieved in mitochondria by retaining a small subset of genes that encode core subunits of the respiratory complexes (Allen 1993, 2003; Allen and Raven 1996; Race et al. 1999). However, neither bacteria nor mitochondria can possibly 'know' in advance which is the correct subset of genes to control redox poise: they were retained in the mitochondria by selection. The ancient mitochondrial genome was gradually whittled away by gene loss and transfers to the nucleus, but if any of the critical genes necessary for maintaining redox poise were lost from the mitochondria, the host cell and its mitochondria would die. Such a gradual whittling process requires stability over evolutionary time that can only be achieved by endosymbiosis. Any other method, such as duplicating the bacterial genome, is liable to be penalised by the heavy selection for small genome size in bacteria. Thus, eukaryotic cells were released from the constraints of a limited bioenergetic surface area by

a rare endosymbiotic event between prokaryotic cells, probably some form of metabolic syntrophy, such as that postulated by the hydrogen hypothesis, which ultimately gave rise to the mitochondria (Martin and Muller 1998). Such endosymbiosis is rare but not unknown in prokaryotes. In contrast, without endosymbiosis, bacteria remained under heavy selection pressure for small size and small genome.

Once mitochondria were established, larger cell size became not just possible but probable. This was because metabolic rates scale allometrically with body mass, with an exponent of less than 1, so that doubling the mass does not double metabolic rate (Kleiber 1961; Hochachka et al. 2003). The metabolic rates of unicellular organisms scale with cell size in a similar fashion, probably owing to the economies of scale in modular operative units (Prothero 1986). Larger cell size is therefore more cost-effective in eukaryotic cells. Larger cells usually have a larger nucleus, with more DNA, giving an optimal karyoplasmic ratio during the cell cycle (Cavalier-Smith 2005). So the selection pressure favouring larger cell size also drove the eukaryotic tendency to accumulate more DNA, and indirectly, greater complexity. Thus, mitochondria made larger size and greater complexity probable, rather than staggeringly unlikely, inverting the constrained world of prokaryotes (Lane 2005).

Biological complexity was only made possible by the energetic and genomic reorganisation emerging from a confederacy of endosymbionts, which ceded most of their powers to central government (the host cell nucleus), but retained flexible regional governance of ATP generation. The hypothesis might be known, if with tongue in cheek, as the federal power hypothesis.

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