Endogenous synthesis of peptidoglycan in eukaryotic cells; a novel concept involving its essential role in cell division, tumor formation and the biological clock

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Abstract. Degradation products of peptidoglycan, the universal bacterial cell wall constituent, were previously found in animal tissues and urine. Reassessment and quantitative analysis of available data lead to an original concept, i.e. that eukaryotic cells synthesize peptidoglycan. We present a model in which this endogenously synthesized peptidoglycan is essential for the processes of eukaryotic cell division and sleep induction in animals. Genes for peptidoglycan metabolism, like those for lysine biosynthesis in plants, are probably inherited from endosymbiotic bacteria, the ancestors of mitochondria and chloroplasts. Corollaries of this concept, i.e. roles for peptidoglycan metabolism in tumor formation and in the biological clock, are supported by abundant evidence. We propose that many interactions between bacteria and eukaryotes are conditioned by their common genetic heritage.

Key words. Biological clock; cell division cycle; diaminopimelate; evolution; FSu; lysine; muramate; muramyl dipeptide; peptidoglycan; sleep muropeptide; tumor.

Introduction

At present, phylogenetic trees which include microorganisms are derived from analyses of nucleotide sequences of ribosomal 16S RNA [102]. However, the most striking differences between the major taxa of the living world, i.e. archaebacteria, bacteria and eukaryotes, are phenotypic. For instance, if bacterial envelopes contain peptidoglycan (PG), and those of certain archaebacteria, pseudomureins, these compounds are absent from eukaryotic cells [32, 40]. It is generally assumed that these differences have their counterpart at the genetic level, each taxon having an assortment of specific genes, not excluding that certain genes, common to all taxa, could have derived from the same ancestral gene.

An example of highly specific genes are those which encode the synthesis of PG, the most distinctive character of the kingdom of bacteria and the target of the most efficient antibiotics, the β-lactams. It is widely accepted that the latter are nearly free of secondary effects on humans, precisely because animal cells are believed to be devoid of PG. Thus, the recently reported presence of apparently small amounts of PG in the urine [49] and in the tissues [84] of higher organisms, was attributed to contamination of the organism by degradation products of intestinal bacterial flora.

We reassess, here, the origin of PG-related molecules in animals and conclude that they cannot but derive from endogenous synthesis. It follows that, for PG, the difference between animals and bacteria becomes quantitative and no longer qualitative. We discuss evidence that bacterial genes introduced into the eukaryotic cells by symbionts, such as the ancestors of the mitochondrion or the chloroplast, were translocated into the host nucleus. We show that PG synthesis can be correlated to two of the most fundamental processes in higher organisms, i.e. cell division and sleep induction. Finally, we propose that bacterial surface components, whose synthesis is encoded in genes inherited by the eukaryotes from the ancestors of the organelles, may play a role in inter- and intra-cellular communication. A recent communication summarized the main points of this contribution [77].

Preliminary considerations

Peptidoglycan metabolism in bacteria

The main features of PG metabolism were obtained from studies on *Escherichia coli* and *Bacillus subtilis*, the paradigm gram-negative and gram-positive bacteria, respectively [74]. They are represented schematically in figure 1, B and C, where three stages can be defined, namely, the synthesis of soluble precursors, their transport across the cytoplasmic membrane and, finally, the polymerization and the maturation phases which end with the release of specific degradation products. PG synthesis requires C 55 undecaprenol and D-amino acids which are obtained via specific metabolic pathways.

The synthesis of the cytoplasmic precursors involves, first, two enzymic activities which convert UDP-N-acetyl-D-glucosamine (UDP-NAG) into UDP-N-acetyl-D-muramate (UDP-NAM). Subsequently, the following amino acids are added sequentially to the free carboxyl group of UDP-NAM by specific so-called adding enzymes: L-alanine (L-Ala), D-glutamate (D-Glu), L,D-diaminopimelate (L,D-DAP) and D-alanyl-D-alanine (D-Ala-D-Ala). Thus, the ultimate cytoplasmic precursor of PG, the UDP-N-acetyl-D-muramyl-pentapeptide (UDP-NAM-pentapeptide) is obtained.

The transport of the precursor across the cytoplasmic membrane requires a lipid carrier, the C 55 undecaprenol, and the activity of two enzymes which mediate the synthesis of C 55-NAG-NAM-pentapeptide. The latter can cross the membrane and reach the periplasmic space.

The polymerization of the glycan chain and the transpeptidation (fig. 2 A) between neighboring chains require the activities of penicillin-binding proteins (PBP). The final stage in rod-shaped bacteria comprises an upwelling of

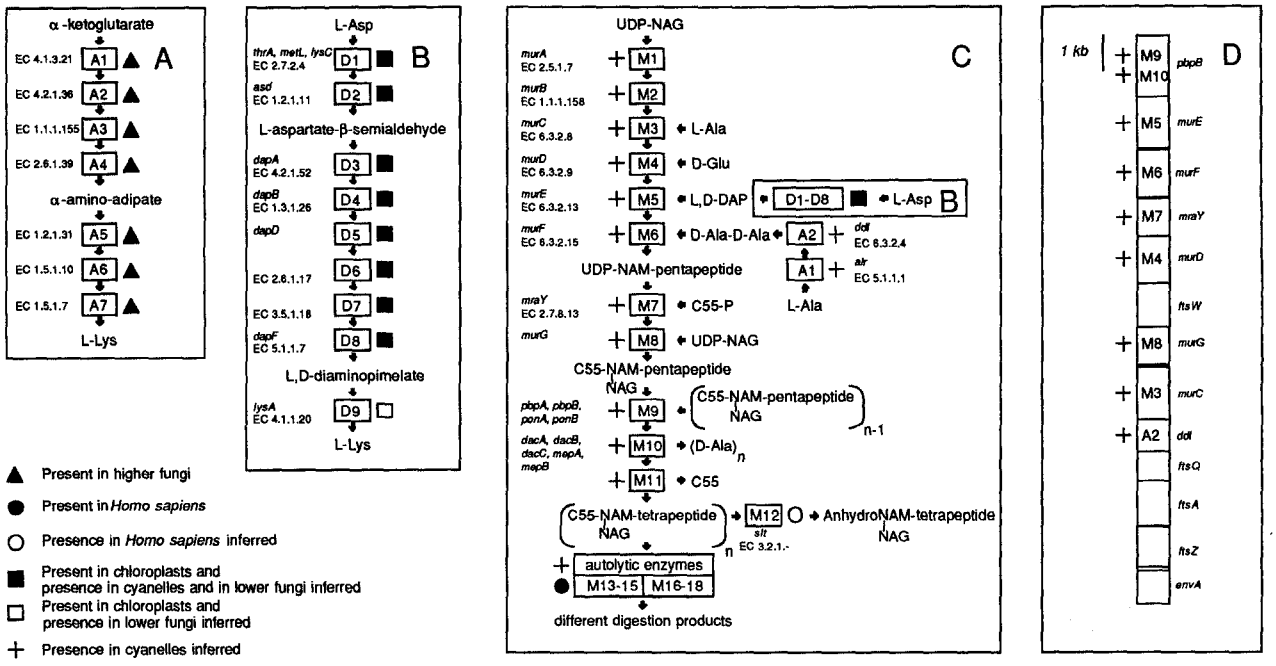

Figure 1. A schematic representation of the peptidoglycan metabolism, of a PG morphoregulon of *E. coli*, and of a fungal lysine biosynthetic pathway. Enzyme commission (EC) numbers are indicated. When known, the *E. coli* gene designation is also shown. Enzymes found in eukaryotic cells are specified. *A* The α-amino adipic acid (AAA) pathway of lysine synthesis[95]. *B* The DAP pathway of lysine synthesis[12]. *C* Formation of the soluble precursors, membrane translocation, PG polymerization and degradation[5,34,35,69]. M12, soluble lytic transglycosylase (transglycosylase); M13, lysozyme (muramidase); M13, β-N-acetylglucosaminidase (glucosaminidase); M15, N-acetylmuramyl-L-alanine amidase (amidase); M16, L,D-carboxypeptidase; M17, D,D-carboxypeptidase; M18; D,D-endopeptidase. *D* The organization of a PG morphoregulon obtained from genetic and nucleotide sequence analyses in *E. coli*[35].

PG[70], i.e. a spreading and a migration to the outer surface of the wall, which is followed by its degradation. The latter is achieved by peptidoglycan hydrolases which include lysozyme, amidase, glucosaminidase and transglycosylase[34] (figs 1 C and 2 A). In rod-shaped bacteria, the synthesis of the septum, followed by daughter cell separation, is a distinct event in the cell cycle[55].

PG-related metabolic activities in eukaryotes

PG and its constituents, muramate, D-Glu, L,D-DAP, and D-Ala, the most characteristic bacterial components, as well as the enzymic activities involved in their synthesis and degradation, have nevertheless been identified in eukaryotes (table 1 and fig. 1, B and C). In general, the complete set of enzymes forming the biosynthetic pathway of lysine, via L,D-DAP, the peptidoglycan specific precursor (fig. 2) was found in plant chloroplasts[10,96]. However, the genes encoding the synthesis of L,D-DAP were not localized in the chloroplast, but in the nucleus. The PG type containing L,D-DAP in position three, present in *E. coli* and *B. subtilis* (fig. 2), has been observed in the cyanelle, the chloroplast of the Glaucocystophyta, *Cyanophora paradoxa* (an alga)[2,43] demonstrating the presence of a complete set of genes required for PG synthesis[69]. Since there is a considerable homology between the genetic maps of the cyanelle and the spinach chloroplast[90], both derived from cyanobacteria[66,91], it is probable that, like the genes encoding L,D-DAP synthesis, the other PG genes are localized in the eukaryotic cell nucleus.

PG constituents have also been found in higher animals. DAP is excreted daily in constant and relatively important amounts in human urine[49]. Tissues of rat liver, brain, and kidney were consistently shown to contain small amounts of muramate[84]. The NAG-anhydro-NAM-L-Ala-D-Glu-L,D-DAP-D-Ala (FSu) (fig. 2 C) was extracted from human cerebrospinal fluid (CSF) as well as being isolated from human urine[58]. Three PG lytic enzymes (figs 1 C and 2 A), i.e. the amidase[60], the glucosaminidase[92], and the lysozyme, were observed in human tissues. Finally, presence of FSu, which requires the activity of the lytic transglycosylase, suggests the presence of the latter enzyme.

Analytical results

Endogenous PG synthesis in eukaryotes

Observations on chloroplasts and in particular on cyanelles (see above) reveal unambiguously that both L,D-DAP and PG can be synthesized by eukaryotes. While their synthesis takes place in the organelles, several if not all of the relevant genes are localized in the nucleus. However, hitherto, presence of PG components in animals has been attributed to contamination by bacterial cell wall catabolites originating from the intestinal flora[49,67,84], to storage of contaminating bacterial PG

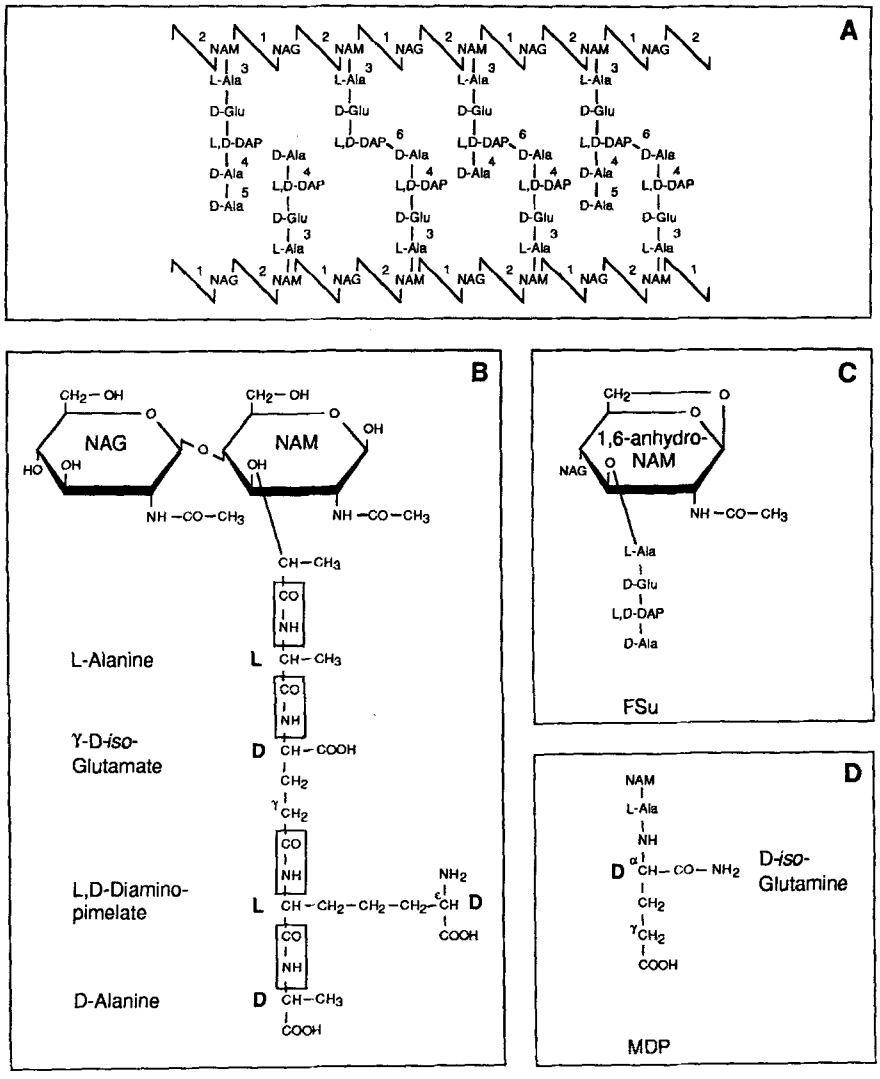

Figure 2. Structure of the peptidoglycan and of related molecules. *A* The cross-linked cell wall structure presented is of the A1γ type[80]. L,D-DAP is the third amino acid of all known purple bacteria (*Escherichia coli, Serratia marcescens*, for example)[80], of all cyanobacteria and cyanelle *Cyanophora paradoxa*[2,18,43,69], of a minor part of gram-positive bacteria (*Bacillus subtilis, Brevibacterium divaricatum, Mycobacterium tuberculosis*, for instance)[14,46,80], and of one minor taxon of the spirochetes (*Leptospira*)[39]. The major part of gram-positive bacteria, certain gram-negative bacteria, among which the majority of spirochetes, have a peptide different from that presented in the figure. Sites at which autolytic enzymes cleave the PG are specified[34]: 1, the lysozyme and the transglycosylase; 2, the glucosaminidase; 3, the amidase; 4, the L,D-carboxypeptidase; 5, the D,D-carboxypeptidase; 6, the D,D-endopeptidase. *B* NAG-NAM-tetrapeptide, the basic unit of PG, obtained after the action of the D,D-carboxypeptidase, D,D-endopeptidase and the lysozyme. *C* FSu, the anhydro-disaccharide tetrapeptide[58] obtained by degradation of PG through the action of D,D-carboxypeptidase, the D,D-endopeptidase and the soluble lytic transglycosylase. *D* The synthetic adjuvant muramyl peptide MDP.

components in tissues[67,84], as well as to bacteria that were engulfed by macrophages and transported into tissues[67,84].

As revealed by the following analysis, these explanations are unsatisfactory. To obtain a more intuitive idea of the quantities of PG involved, we will express them in terms of *E. coli* cell wall equivalents (ECWE). Since in minimal medium one ECWE contains $3.5 \cdot 10^6$ molecules of L,D-DAP[100], the 3.75 μmoles of DAP eliminated daily in human urine[49] are equivalent to the PG mass of $6.5 \cdot 10^{11}$ *E. coli* cells. To examine if this amount of PG could have been degraded and absorbed in the digestive tract in 24 h, we will summarize the relevant data. The absorption of amino acids is confined to the small bowel, and is absent in the colon[30], which contains almost all of the 10^{14} bacteria[79] forming the human intestinal flora. The bacterial concentration in the small bowel forms a gradient beginning with 10^1-10^2 cells/ml in the duodenum, and increasing along the ileum to reach, in its distal segment, about 10^6-10^7 cells/ml[17]. This distribution suggests a high through flow rate and a relatively slow bacterial multiplication. Since there is evidence in favor of slow growth of all intestinal bacteria during their passage through the small bowel[28], only cell wall released by turn-over[70] can be degraded and possibly absorbed. It is likely that walls of only half of the bacteria localized

Table 1. Inventory of enzymes and compounds related to PG metabolism which were found in eukaryotes

Chemistry	Diaminopimelate in human urine: 714 µg (or 3.75 µmol) of DAP per day[49].
	Muramate in rat brain and kidney: 50–100 pmol/g fresh weight[84]. Muramate in rat liver: 100–150 pmol/g fresh weight[84].
	Sleep muropeptide (FSu) in urine and in cerebrospinal fluid (CSF) of sleep-deprived animals: 30 µg purified from 5000 l of human urine[67].
Morphology and chemistry	The cyanelle of *Cyanophora paradoxa*[2] is surrounded by a PG cell wall. This PG was found to be analogous to that of cyanobacteria[18,80], from which the presence of the entire PG metabolism is inferred[69].
Enzymology	Biosynthesis of lysine, derived from aspartate via the DAP pathway, in chloroplasts[10,96].
	Autolytic enzymes found in humans: N-acetylmuramyl-L-alanine amidase[60]; β-N-acetylglucosaminidase[92]; soluble lytic transglycosylase, inferred from the presence of FSu; lysozyme.
Tertiary structure	Cross reactivity of antibodies against interleukine 1 and against MDP[11]. Serotonin and PG compete for the same macrophage receptor[42].
Physiology	Endogenous production of interleukin-1 by macrophage is induced by MDP[48]. Pyrogenicity of interleukine-1[11], MDP[67], and FSu[46]. Mitogenicity of MDP or PG in B lymphocytes[1,20]. Induction of slow wave sleep by MDP[67], FSu[46], and interleukin-1[47].

in the ileum contain L,D-DAP, while the remainder contain lysine, ornithine or lanthionine instead of L,D-DAP[51,61,94]. We will assume that the small bowel of a 1-l content is emptied every 24 h, that the concentration of DAP yielding bacteria is $5 \cdot 10^6$ throughout the small bowel, that the total cell wall released through turn-over, corresponding to about 10% of the total bacterial cell wall[70], is fully degraded, and that all the resulting amino acids are absorbed. Thus, in 24 h, the amount of DAP absorbed would be equivalent to $5 \cdot 10^8$ bacteria ($0.1 \cdot 1000 \cdot 5 \cdot 10^6$), which is negligible compared to the $6.5 \cdot 10^{11}$ ECWE measured in urine[49]. Even this calculated figure is likely to be overestimated. Indeed, to our knowledge, animals are devoid of peptidases capable of cleaving L,D, iso-L, and D,D peptide bonds, present in the muropeptide (fig. 2B). Therefore, the DAP in human urine cannot originate from the intestinal flora. This conclusion is supported by the observation that the amount of DAP excreted in urine of different individuals is fairly constant[49], despite possible important quantitative and qualitative variations in their intestinal flora[16].

The example of cow is also revealing. The 180-l rumen of a 600-kg cow[36] corresponds to a culture of $2.2 \cdot 10^{10}$ bacterial/ml[93], half of which contain L,D-DAP in their walls. Although possible degradation products of this colossal bacterial population transit subsequently through the small bowel, where amino acid adsorption takes place, the amount of DAP per kg, excreted in cow urine, was only three times higher than in man[49]. The presence of muramate and in particular of FSu in the brain and in the CSF, respectively[67,84], is even more difficult to reconcile with an exogenous bacterial origin. It is improbable that PG degradation products could have been introduced into the CSF by transport of bacterial wall debris from the blood plasma. First, the CSF is protected by a highly selective barrier, even towards low molecular weight components[72], and second, sleep induction in animals by MDP (see below and fig. 1D) requires 10^4-fold higher concentrations when MDP is administered intravenously when compared with direct injection into the nervous system[67]. Finally, it is unlikely that the tissue concentration of molecules as potent as muropeptides could depend exclusively upon absorption of metabolic breakdown products of the intestinal flora whose PG composition is subject to variation[16] (fig. 1). The most obvious and probably the unique solution of this paradox is that genes encoding PG metabolism or part thereof have been retained not only in lower eukaryotes and plants, but in the chromosomes of higher animals, and that molecules derived from PG, which are found in animal and human urine and tissues, are synthesized endogenously.

Role of endogenously synthesized PG in eukaryotes

Several lines of evidence suggest that PG plays a role in eukaryotic cell division (table 1). In particular, PG degradation products are pyrogenic, as well as mitogenic. We propose to establish a quantitative relationship between the measured amounts of PG components and cell division on the basis of the following observations. PG and L,D-DAP synthesis in plants and *C. paradoxa* are associated with the chloroplast and the cyanelle, organelles of bacterial origin. Treatment of animals with chloramphenicol, whose target is the mitochondrial 70S ribosome[27], reduces by half the amount of DAP excreted in animal urine[49], while chloramphenicol treatment of humans is known to lead to medullar aplasia[50]. Compatible with the foregoing is the view that PG synthesis is confined to the tiny fraction of growing and dividing cells, and that it takes place in mitochondria. We propose that the amount of PG synthesized by each mitochondrion is the same as that of an *E. coli* cell of equivalent volume. Release of PG components to the urine is consistent with its enzymatic degradation.

With this in mind, we will compare cell division and PG synthesis in rat hepatocytes. 1 g of liver contains $1.69 \cdot 10^8$ hepatocytes of 4940 μm^3, each harboring 1665 mitochondria [98]. The diameter of the latter is comparable to that of an *E. coli* cell. The volume of a mitochondrion in a resting cell is 0.7 times that of an *E. coli* [15, 98]. Before mitosis, the total mitochondrial volume, and thus the number of mitochondria per cell, is twice that of resting cells [71]. Therefore, a hepatocyte about to divide would contain 3330 mitochondria. The reported mitotic index of rat hepatocytes is $5 \cdot 10^{-5}$ [25], and the average number of cells undergoing mitosis in one gram of liver is $8.45 \cdot 10^3$ ($5 \cdot 10^{-5} \cdot 1.69 \cdot 10^8$); thus, with the given assumptions, the total quantity of PG in these cells would be $1.97 \cdot 10^7$ ECWE ($8.45 \cdot 10^3 \cdot 3330 \cdot 0.7$ ECWE). This figure is in remarkable agreement with the $2.2 \cdot 10^7$ ECWE, corresponding to the 125 pmoles of trichloroacetic acid extractable muramate found [84] per g of rat liver. Thus, like in bacteria, PG synthesis and cell division in eukaryotes appear to be coupled. Cell division in bacteria corresponds to an accurately timed massive incorporation of PG into the septum [55]. Generally, in the absence of PG synthesis, regular bacterial division does not take place, as shown by the irregular growth and division of bacterial protoplasts and L-forms [74]. It is therefore possible that synthesis of septal components is triggered by a PG-related metabolism, and that it is precisely the incorporation of such a system into eukaryotic cells (see discussion) that has conferred on the latter the potential for regular cell division and multiplication.

Our analysis is compatible with the cell division cycle model presented in figure 3. Since PG degradation products are mitogenic (table 1), onset of PG metabolism must precede mitosis. We propose that a pulse of PG is synthesized in mitochondria of cells whose division is programmed. Its amount per cell would be that present in the mass of *E. coli* cells whose total volume is equivalent to that of all mitochondria contained in the dividing cell. The synthesis is followed by PG degradation into muropeptide which involves autolysins and, more specifically, the lytic transglycosylase. Finally, release of muropeptide to appropriate targets would trigger mitosis.

There is no morphological evidence, however, of mitochondria in animal tissues being endowed with cell wall. Two explanations may be considered. First, PG is confined to mitochondrial invaginations and thus does not appear as a typical bacterial wall layer. Second, its apparently ephemeral presence in eukaryotes and the rareness of dividing cells might have precluded its visualization in cell sections. Finally, PG synthesis and degradation might be quasi-simultaneous.

Comparison of the amounts of DAP excreted by mammals [49] and their life expectancies, is also suggestive of a role for PG in cell division. Indeed, the product of the life expectancy and the amount of DAP excreted daily (in nmoles/kg) for man, cow and swine [49], as well as rat, is substantially the same (table 2). Considering that life expectancy decreases with increasing mitotic index, as observed on swine and cow leukocytes [88], it follows that the quantities of excreted DAP are positively correlated to the mitotic index. The amount of excreted DAP [49] does not seem to be related to the composition and the titre of the intestinal flora of different animals [19].

Correlation of the quantity of muramate or of DAP with the number of dividing cells gives a new significance to these easily measurable parameters. Their assay should provide direct information on global rate of cell division and regeneration of an organism or of a given tissue, under various normal or pathogenic conditions. Assuming that the average dividing human cell contains an equivalent of 800 mitochondria (see above), the

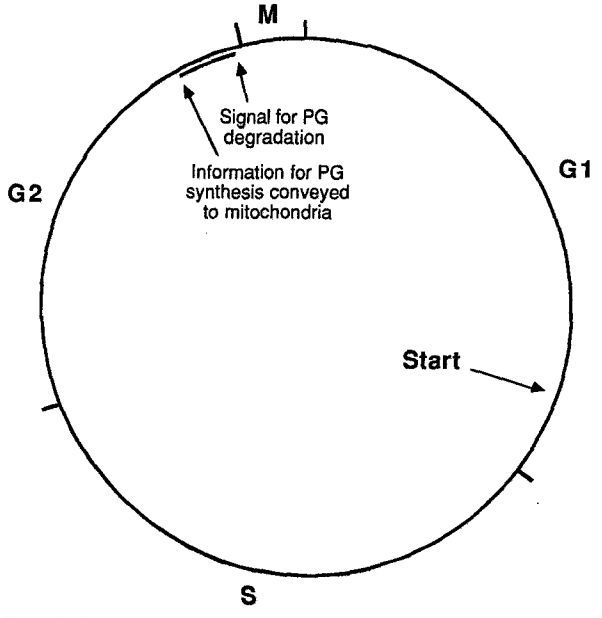

Figure 3. PG metabolism in the eukaryotic cell division cycle. PG synthesis takes place in cell undergoing a division cycle [64] and is confined to their mitochondria. It is initiated before the onset of the mitosis and yields about 0.7 equivalents of *E. coli* cell wall per mitochondrion. Subsequently, PG is degraded, released into the cell where it participates in nuclear and cell division processes, and finally, excreted from the cell, G1, S, G2 and M correspond to gap1, DNA synthesis, gap2 and mitosis of a hypothetical 24-h cell division cycle.

Table 2. Excretion rates of L,D-DAP in urine as a function of life expectancy of different organisms

Animal	Life expectancy (years)	L,D-DAP excreted in 24 h (nmoles per kg animal)	Life expectancy times L,D-DAP excreted in 24 h per kg animal (year · nmole per kg)
Man	80	50	4000
Cow	25	150	3750
Swine	10	350	3500
Rat	3	1200[a]	3600

[a] Calculated by assuming a mean muramate content in rat of 50 nmoles per kg and an average mitosis time of 1 h. Thus, the amount of muramate excreted per kg tissue in 24 h is 1200 nmoles (50 · 24).

3.75 μmoles of DAP excreted daily in urine correspond to the regeneration, in an adult, of $1.2 \cdot 10^9$ cells ($9.2 \cdot 10^{11}/800$), i.e. 0.012% of the 10^{13} cells contained in a human body[79].

PG and sleep induction

Search for muramate containing compounds in sleep-deprived animals yielded invariably NAM, alanine, glutamate and L,D-DAP in a molar ratio roughly comparable to that of FSu[58, 67]. Intracerebroventricular administration of 1 pmole of FSu to a rabbit increased the proportion of slow wave sleep from 40 to 70%[46]. Intracerebroventricular infusion of FSu into cats also improved the quality of sleep[67]. The effect was produced by FSu either extracted from human urine or obtained by biodegradation of the cell wall of *Brevibacterium divaricatum*[46]. Although this remarkable series of experiments leaves no doubt that FSu is a natural sleep-inducing agent, the possibility of its endogenous synthesis has been hitherto discarded.

We will examine these observations in the light of our concept of endogenous PG synthesis (see above) and the well established role of the hypothalamus, whose lesions permanently prevent slow wave sleep. By analogy to the model described here above, which associated PG synthesis to the amount of mitochondria in appropriate cells, we can calculate the number of cells required to synthesize the total amount of muramate found in the 2-g[38] rat brain, i.e. 150 pmoles (2×75 pmoles)[84] or $2.64 \cdot 10^7$ ECWE. We assume that brains were prepared during the morning, i.e. following the sleep induction of the rat, which was triggered by a pulse of PG synthesis. Hypothalamus cells being comparable in size to hepatocytes, it follows that the total amount of muramate contained in the brain of a sleeping rat was synthesized by about 10000 cells [$2.64 \cdot 10^7/(3330 \cdot 07)$], which is in fair agreement with the estimated number of neuro-secretory cells of the rat hypothalamus[45]. However, while inducing division in hepatocytes, liberation of PG degradation products in the brain is accompanied by sleep induction, but not by cell division. This could be explained by the absence from brain cells of some other cell division component.

Discussion

The arguments developed above reveal that eukaryotic cells are capable of endogenous PG synthesis, since quantitatively PG constituents, such as L,D-DAP and muramate, found in animal urine and tissues, cannot originate from the intestinal flora. Furthermore, there is genetic evidence for PG genes in eukaryotes. First, an *E. coli dapA* mutation (fig. 2B) was complemented by maize cDNA[26], and second, amino acid sequence homology was observed between the FtsA protein, part of the complex involved in the synthesis of septal PG[78], and two eukaryotic cell division cycle proteins (CDC)[73]. PG synthesis plays a key role in cell division and sleep induction. At an early stage of mitotic division, information for PG synthesis would be transmitted from the nucleus to mitochondria of cells programmed to divide. The amount of PG synthesized per mitochondrion would be equivalent to that associated with a gram-negative bacterium of comparable size and shape. Subsequent degradation of PG into muropeptide – probably FSu – and its transport to the appropriate site(s) would trigger cell division, which will in turn be followed by excretion of FSu, and its elimination via the urine. PG found in the brain, probably also synthesized as a pulse in the hypothalamus, would subsequently trigger, more or less directly, slow wave sleep. We propose, below, an account of the origin of PG metabolism encoding genes present in eukaryotes, and discuss the most evident implications of our model in tumor formation and the biological clock.

Origin of the PG encoding genes in eukaryotic cells

The serial endosymbiosis theory[56] of bacteria, archaebacteria and eukaryotes, provides a model by which a contemporary eukaryotic cell endowed with organelles was obtained. The partners (fig. 4) whose endosymbiosis led to the eukaryotic cell were probably archaebacteria related to *Thermoplasma acidophilum*[82] on the one side, and ancestors of cyanobacteria and purple bacteria on the other side.

The former partner, a poor grower which apparently undergoes division through budding[83], is limited in its metabolic activities, being, for instance, devoid of the Krebs cycle. Like the eukaryotic cell cytoplasm, from which acetyl-CoA is exported into mitochondria, *Thermoplasma* excrete acetate. Absence of cell wall, in this organism, is associated with a facility to engulf other microorganisms and establish symbiosis[82]. The bacterial partners, much more evolved than eukaryotes or archaebacteria[97], are characterized by highly efficient genetic organization and metabolism. For instance, cyanobacteria, the ancestor of chloroplasts, are capable of photosynthesis, while purple bacteria, related to mitochondria[29, 103], possess the Krebs cycle. In general, per unit biomass, the metabolic potential of bacteria is about 100-fold higher than that of cells of multicellular higher organisms.

Organelles of bacterial origin possess various proportions of their ancestral DNA. The size of the chromosomes of mitochondria and chloroplasts is between 1 and 10%[3, 44], and between 10 and 100%[3, 44, 90] of that of their respective putative ancestors, an α-purple bacterium, and a cyanobacterium. Bearing in mind that bacterial genes encode highly efficient metabolic processes and that de novo generation of a gene is a very rare event[4], it would be surprising if such genes were discarded upon endosymbiosis. It is more likely that eukaryotes were, and still are, endowed with a mechanism allowing translocation of symbiotic bacterial genetic entities into the host chromosome and, possibly, vice-versa. First, most of the mitochondrial constituents were shown not

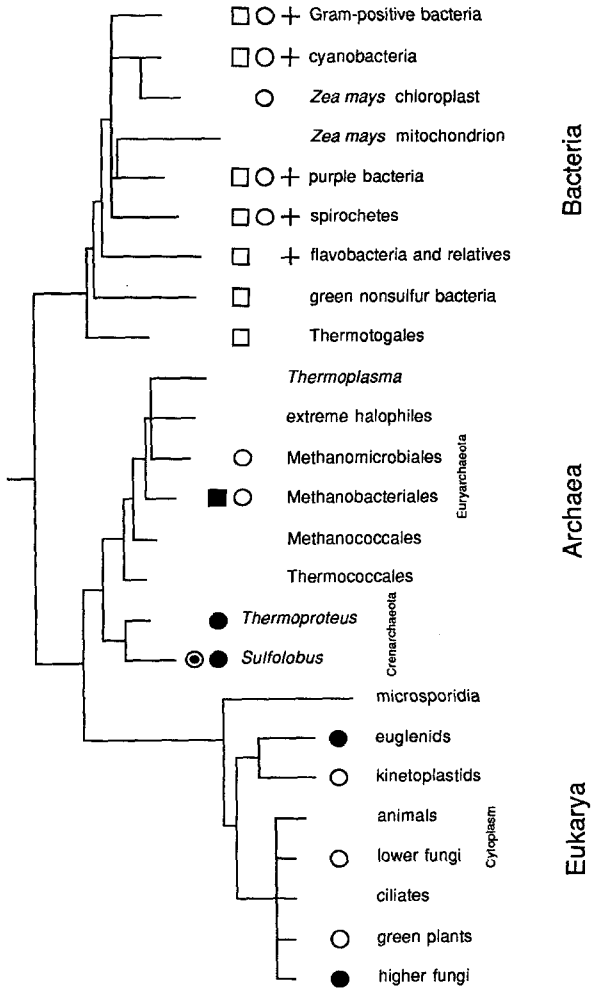

Figure 4. The phylogenetic tree of cellular organisms, obtained from analyses of 16 and 18S RNA sequences, is based on Woese et al.[102], and completed with Pace et al.[65] and Woese[101]. The position of *Thermoplasma* is derived from Woese[102a] and that of eukaryotes from Knoll[43a]. Positions of currently defined kingdoms of bacteria, archaebacteria and eukaryotes are indicated. The nature of the cell wall[32,40] and that of the lysine biosynthetic pathway[7,8,12,23,41,68,76,95] of each taxon are indicated. DAP pathway is confirmed by the presence of L,D-DAP in the third position of the muropeptide[18,39,80]. □, Peptidoglycan; ■, pseudomurein; +, DAP used as the third amino acid in the peptidoglycan; ○, DAP pathway; ◉, evidence for DAP decarboxylase activity[41] (fig. 2); ●, AAA pathway. The symbols (biological tracers) were attributed to the whole taxon when the character was shown to be present in at least one species. Bacteria form a homogeneous taxon, which possess a peptidoglycan cell wall and, like chloroplasts, derive their lysine via DAP. Archaebacteria and eukaryotes are heterogeneous groups endowed with either the AAA or the DAP (fig. 1, A and B). It is possible that like in kinetoplastids[95] and plants the lysine biosynthetic pathway in lower fungi is confined to their organelle. Thus, it would appear that the distribution of the lysine pathway follows the eocyte phylogenetic tree and not the archaebacterial one[72a]. More generally, using lysine pathways as phylogenetic markers, it would appear that nucleated urkaryotes[101] were already polyphyletic, their cytoplasm originating from an eocyte, affiliated to archeabacteria, and their nucleus from an organism related to the true eukaryotic branch.

to be encoded by the genome remnant localized in the organelle[3], and second, more recent experimental evidence in favor of fast rate of exchange of genetic material between chromosomes of *Amoeba proteus* and a so-called x-bacterium, engulfed by endocytosis, was provided[37]. Thus, parts of the genome of bacteria which have entered endosymbiosis, including over 50 genes which encode the PG metabolism (fig. 1, B and C), were probably incorporated into the host's chromosomes and adapted to its expression mechanisms. This could account for the potential of eukaryotic cells to synthesize PG.

PG and tumor formation

The proposed model predicts that the inhibition or the derepression of endogenous eukaryotic PG metabolism would generate disturbances of the cell division cycle. It was suggested that the latter lead to tumor formation[64]. Evidence correlating the presence of PG degradation products, and the possible inhibition of PG synthesis with cell division and tumor formation provide some support for this claim.

A mitogenic effect of PG degradation products has been reported[20]. Prolonged periods of daily low fever accesses, associated with early stages of cancer[89], were accompanied by excretion in urine of pyrogenic substances[86]. Although substances like interleukin, known to be pyrogenic, have been detected in the urine, it is possible that the uncharacterized substance was FSu, whose overproduction, according to our model, would be associated with an increased mitotic index. Thus, measurement of DAP in the urine would allow early detection of cancer development, which, at this stage, might be controlled by administration of products interfering with PG synthesis and (or) degradation. Finally, the tracheal toxin of *Bordetella pertussis*, a compound identical to FSu, is reported to inhibit DNA replication[99] and thus perturbs the cell division cycle.

There is also evidence that PG degradation products and their specific nature can modulate the response of the organism to tumor cells. For instance, therapeutic stimulation of macrophage activity by treatment with BCG (*Bacillus* Calmette-Guérin), the attenuated *Mycobacterium tuberculosis* or by MDP, suggests that cells with derepressed muropeptide synthesis 'attract' macrophages[6,52,53,87]. The latter[21,85], as well as lymphocytes[21,22] were found to possess PG receptors. Therefore, cells with an increased division rate, accessible to macrophages of an immunocompetent host, tend to be eliminated. However, at present, stimulation of the immune response by MDP alone, or with lipopolysaccharide, which is even increased in presence of antibody[53], is attributed to a nonspecific effect. Furthermore, remission of certain tumors has been observed in patients suffering from acute bacterial infections[13]. It was argued that Coley's treatment with supernatants of a mixed bacterial culture, comprising *Streptococci* at high concentrations and killed *Serratia marcescens*, led to a significant increase in the life expectancy of patients suffering from a variety of malignant tumors. This effect might have been due to defense stimulation like that induced by MDP or BCG or to release of tumor-specific toxins. However, a massive release or injection of PG-related compounds acting as competitive inhibitors to the mito-

sis signal could also be implicated. More recently, interference of *Arthrobacter* cell wall degradation components with the development of the Kaposi's sarcoma was reported[63]. Finally, inhibitory action of bacterial glucosaminidase on both the mitogenic effect of, and the immune response to, concanavalin A in mice macrophages[92] suggest that specific PG degradation products play a role in eukaryotic cell division.

Other evidence suggests that inhibition of PG synthesis prevents cell multiplication. For example, treatment with chloramphenicol, which interferes with the functioning of mitochondrial 70S ribosomes and thus, according to our model, prevents synthesis of PG forming enzymes, is known to induce medullar aplasia[50].

Should derepression of muropeptide synthesis be related to tumor formation, growth inhibition of tumor cells could be attempted by localized treatment with antibiotics to which the cytoplasmic membrane is permeable. Conversely, the elimination of muropeptide-producing pathogens induced by a systemic antibiotherapy could lead to an attenuated immune response, due to absence of macrophage stimulation, and provide more favorable conditions for tumor development. Again, assay for muramate or DAP in normal and tumor tissues should provide information on the relative rates of cell division.

PG and the biological clock

FSu, the potent sleep-inducing agent, was found in animal brain tissue at concentrations sufficient to induce sleep[58,67]. Therefore, its synthesis is probably under the control of the biological clock. By analogy to PG synthesis regulation in bacteria (fig. 1, B and C), we can assume that, in higher organisms, PG biosynthesis or biodegradation are accurately timed and triggered like, for example, the synthesis of the bacterial septum[55]. The time of activation of the relevant timer-switch, possibly translocated from the organelle's ancestor into the chromosome (see above), could be determined by its position on the chromosome and the speed of either some kind of DNA processing or of a more elaborate sequential process. For example, under given conditions – exponential growth at 37 °C in glucose minimal medium – the cell cycle of *E. coli* lasts about 60 min, while the replication of the $4.7 \cdot 10^3$ kbp chromosome[44], which is initiated immediately following cell division, is achieved in 40 min[9]. If we assume that in higher organisms a process analogous to that in bacteria takes place at the same speed, we can calculate that FSu release will be triggered with a periodicity of about 24 h for a chromosome of $170 \cdot 10^3$ kbp. This figure fits well with the size of human chromosome, i.e. $50–263 \cdot 10^3$ kbp[62]. The foregoing model, named

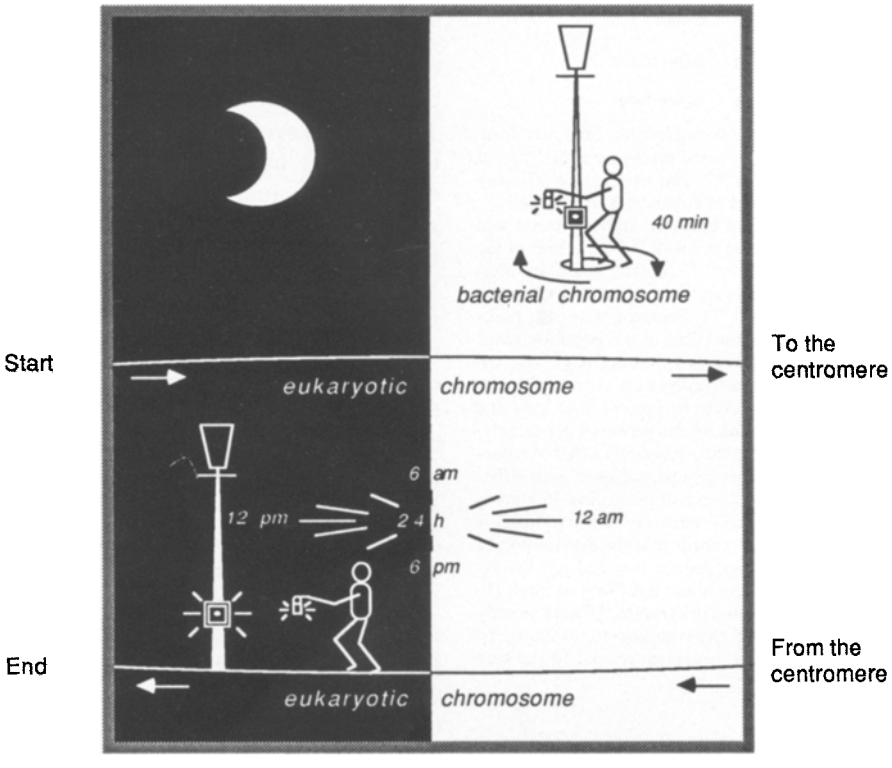

Figure 5. Representation of the biological clock. The model named KANT – 'Knockout Administered by the Nucleic Traveller', in memory of the clock regulated life of Immanuel Kant [36a]. A wanderer walks along a path (the linear eukaryotic chromosome represented as an open circle) at a constant speed following a circadian rhythm. At a given point (normally at night fall), he reaches and turns on the lamp, by pressing on a timer-switch the signal for PG synthesis and the release of sleep muropeptide which administrates the knockout. Thus, the organism is put asleep for a predetermined period of time. For comparison, the corresponding bacterial path, a circular one, 50 times shorter, is represented. In this case, the wanderer, moving at the same speed as in eukaryotes, triggers the timer-switch for septation and cell division. Translocation of this bacterial switch into the eukaryotic chromosomes would have offered to eukaryotes the possibility to establish the circadian rhythm.

KANT is schematically represented in figure 5. Study of FSu, as well as of the system by which its synthesis and release are regulated, could find applications in medicine or, for instance, in jet-lag correction.

Cell division and sleep induction, two of the possible roles of PG components in eukaryotes, would have in common a similar or identical signal molecule, a muramyl peptide. Like bacterial PG synthesis, that of FSu seems to be sensitive to cycloserine, an antibiotic inhibiting the synthesis of UDP-NAM-pentapeptide, the ultimate soluble PG precursor (fig. 1). Indeed, it is possible that the nearly unrestricted access of cycloserine to the CSF [50], and the resulting inhibition of FSu synthesis may account for symptoms of hypomaniacal delirium in cycloserine-treated patients [50]. The cell division and the sleep induction molecules may also have an additive effect. For instance, the somnolence of newborns may result from the increased concentration of the cell division muropeptide in the organism due to accelerated cell multiplication, while the fever accompanying sleep induction in patients suffering from the Hodgkin's disease suggests that an already high level of FSu due to abnormal lymphocyte production is amplified by sleep associated FSu synthesis to the extent of being pyrogenic. Finally, under certain physiological conditions, cell division and sleep induction exhibit the same periodicity. Indeed, cell division in tissue cultures, in particular of the HeLa cells [71] or the first divisions in the human fetus [24], as well as heat generation associated with sleep induction [67], occur every 24 h.

Conclusions

The capacity of eukaryotic chromosomes to acquire bacterial genes by translocation from organelles provides an explanation for the bacterial origin of many of the highly efficient eukaryotic functions. For example, lysine, an essential amino acid, is synthesized in chloroplasts by a cyanobacterial complex through decarboxylation of DAP (figs 1 B and 4). The cellulose biosynthetic complex present in α-purple bacteria [75] was probably acquired by plants through endosymbiosis with an ancestral relative of the mitochondrion. The undulipodium was inherited from the axial fibrils of spirochetes [33]. The fact that microbial antigens cross-react with similar domains of the plasma membrane of mammalian cells [57] is an indication of common origin. Observations on cross reaction between blood group determinants and bacterial polysaccharides have recently opened the question of possible genetic exchange between bacterial and eukaryotic genomes [54]. Even genes of bacteriophages of purple bacteria, related to the *E. coli* phage T4, were apparently retained by eukaryotes. Indeed, amino acid sequence homologies between the intron in the T4 thymidylate synthase gene and that in the NADH-dehydrogenase subunit I gene of *Neurospora crassa* [59], as well as structural homologies between T4 and animal lysozyme [31], have been observed. Recently, endosymbiosis between phage infected bacteria and a Cryptophycea was reported [81].

As suggested here above, degradation products of PG, a bacterial surface component, are involved in eukaryotic intra- and inter-cell communication. However, the question of the fate of bacterial communication systems, including surface structures, encoded by genes retained by eukaryotes can be raised in more general terms. It is possible that the bacterial surface components – fimbriae, S-layers, capsules, cell wall components, and flagella – which were encoded by the organelle's ancestor, are involved in eukaryotic cell communication, as well as in recognition of, and communication with, pathogenic or other microorganisms. Investigators studying the interference of a pathogen or a virus with the function of a higher organism should be aware of the possibility of interactions at the level of communication signals. For instance, the nature and the extent of PG processing by autolysins, which modifies the PG degradation products, has been cited as playing an important role in the severity of a bacterial infection [34].

Considerations developed above imply that understanding of bacterial and bacteriophage genetic regulatory units that have been retained by higher organisms could be achieved with related, free-living bacterial species. We believe that it will be highly appropriate to speed up the sequencing of the chromosomes of the paradigm bacteria, *E. coli* and *B. subtilis*, while setting up that of the chromosomes of an α-purple bacterium (*Paracoccus*), a spirochete, a cyanobacterium, the putative ancestors of eukaryotic organelles, and a *Thermoplasma*-like archaebacterium.

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