

The modern concept of the procaryote

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Running title: Prokaryote concept

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2           In their seminal work, Stanier and van Niel (47) proposed that bacteria were  
3 distinguished from other forms of life, including viruses, protists, fungi, algae, plants and  
4 animals, by their procaryotic cell structure. They defined the procaryotic cell by three  
5 major criteria: the absence of internal membranes that compartmentalized the nuclear  
6 material and the enzymatic machinery for respiration and photosynthesis, nuclear  
7 division which occurred by fission and not mitosis, and the presence of peptidoglycan in  
8 the cell wall. They also recognized that procaryotes possessed enormous diversity,  
9 similar in scope to that of eucaryotic protists, and further proposed that the procaryotes  
10 represent a distinct mode of evolutionary diversification parallel to that found in the  
11 eucaryotes (47). Thus, the procaryotic:eucaryotic dichotomy was founded upon the  
12 recognition of two very different types of cellular organisms and not the phylogenetic  
13 relationships between them.

14           Recently, five major criticisms of the concept of the procaryote have been  
15 proposed (36,55): it fundamentally contradicts the three domain model of life, the  
16 procaryotes are not monophyletic, the procaryotes are defined by negative characteristics,  
17 the term procaryote “sustain[s] the concept that procaryotes evolved into eucaryotes”, and  
18 the term is imprecise. As shown below, these criticisms are misstatements of the original  
19 proposal and modern descriptions of the concept or otherwise erroneous (47, 27). In fact,  
20 since its original proposal in 1962, the experimental evidence for this concept has been  
21 enormously enriched.

22           Today the concept of the procaryote includes a much greater understanding of the  
23 molecular basis for the differences between procaryotic and eucaryotic cells. In addition,  
24 it recognizes the antiquity, abundance, and diversity of procaryotes. Procaryotes likely

25 dominated life on the early earth for over a billion years prior to the appearance of  
26 eucaryotes. Today, the biomass of the procaryotes is comparable to that of eucaryotes.  
27 The procaryotes are also extremely diverse, and representatives of two ancient domains,  
28 the Bacteria and Archaea, are common today. Each domain includes organisms with  
29 many different metabolic and physiological capabilities, and the number of species is  
30 correspondingly so large that it has never been estimated accurately.

31 **The procaryotic cell.** The cellular organization of procaryotes is of fundamental  
32 importance to their physiological and biochemical processes, and their differences with  
33 eucaryotes are well described (27). Three features are especially relevant. One, nuclear  
34 membranes are absent, which allows coupled transcription and translation (13, 33).

35 Because the DNA is not segregated to the nucleus, it is also possible to regulate  
36 transcription with repressors and activators that bind metabolites. In this sense,  
37 transcriptional regulation is further coupled to metabolism. In the eucaryotes, the major  
38 metabolic processes occur in the mitochondria, chloroplast, and the cytoplasm and are  
39 isolated from transcription in the nucleus.

40 Two, procaryotic cells are usually smaller than eucaryotic cells. There are some  
41 notable exceptions. The sulfur-oxidizing bacterium *Thiomargarita* has a diameter up to  
42 750  $\mu\text{m}$  (44), which is larger than many protists. The eucaryotic marine picoalgae, which  
43 are 1-2  $\mu\text{m}$  in diameter, are similar in size to many procaryotes (41). In spite of this  
44 diversity, size remains an important distinguishing characteristic (58). Size establishes  
45 the surface to volume ratio of the cell, which limits the rate and type of nutrient uptake.  
46 It also allows for rapid diffusion of small molecules and proteins throughout the entire  
47 cell, which provides a mechanism for coupling metabolism and regulation.

48           Three, the cytoplasmic membrane is multifunctional in procaryotes and represents  
49 the defining structure of the cell. A proton motive force is generated on the cytoplasmic  
50 membrane by respiration, photosynthesis, or ATP hydrolysis to empower key cellular  
51 processes such as ATP biosynthesis,  $\text{NAD}^+$  reduction by reverse electron transport,  
52 nutrient uptake, motility, and secretion. Procaryotes utilize membrane transporters on the  
53 cell surface to assimilate nutrients dissolved in their environment. In many procaryotes,  
54 the cytoplasmic membrane possesses a complex topology composed of lamellae, tubules  
55 or other cytoplasmic intrusions (27). In contrast, the cytoplasmic membrane of  
56 eucaryotes is very different in structure and function. Eucaryotes commonly take up  
57 particulate material by phagocytosis, a process that does not occur in procaryotes.

58           **Evolution of procaryotes.** Geochemical and fossil evidence indicates that life on  
59 earth is at least 3.5 billion years old (1, 42, 43). While the form of ancient microfossils  
60 resembles that of modern procaryotes, there is little additional evidence in the fossil  
61 record for their molecular nature. However, by 2.5 billion years ago, there is evidence  
62 for abundant procaryotic life, including widespread microfossils and stromatolites or  
63 fossilized microbial mats, and major signatures of biological processes in the  
64 geochemical record, such as depletion of inorganic carbonates for  $^{12}\text{C}$  and deposits of  
65 complex organic carbon enriched in  $^{12}\text{C}$  (42). By this time, the oxygenation of the earth  
66 was well underway, and it is likely that oxygenic photosynthesis was fully evolved within  
67 the Bacteria (2, 9). Because of its biochemical complexity and limited distribution, it is  
68 likely that oxygenic photosynthesis evolved fairly late in the history of the procaryotes.  
69 Thus, procaryotes very similar to modern organisms were probably abundant on earth 2.5  
70 billion years ago. In contrast, the first fossils of clearly eucaryotic organisms appeared

71 about 1.8 billion years ago (24). Similarly, analyses of the molecular diversity within the  
72 modern eucaryotes suggest that this group began to diversify about 1.1-2.0 billion years  
73 ago (8, 17). Thus, it is likely that the eucaryotes only evolved after the procaryotes had  
74 obtained their modern complexity.

75         Although not stated explicitly, it is likely that Stanier and van Niel (47) believed  
76 that the procaryotes comprised more than one major phylogenetic lineage. They  
77 recognized that procaryotic diversity was comparable to that of the eucaryotes, as  
78 evidenced by various photosynthetic and chemotrophic metabolisms; unicellular,  
79 multicellular and coenocytic lifestyles; and cellular multiplication by binary fission,  
80 budding or the formation of conidia. Moreover, they proposed that the procaryotes  
81 represented one of two parallel forms of evolutionary diversification, with the second  
82 form being the eucaryotes. At the time, the eucaryotes were believed to be comprised of  
83 multiple lineages, so this dichotomy implied that the procaryotes were also comprised of  
84 more than a single lineage. Originally discovered based upon comparisons of 16S  
85 ribosomal RNA sequences, the presence of two ancient lineages or domains of the  
86 Bacteria and Archaea within the procaryotes is now well established (Figure 1; 56).  
87 Fundamental differences in the molecules required for information processing systems,  
88 such as translation, transcription and replication, further support the early emergence of  
89 these domains (28, 32).

90         **Procaryotic domains.** In addition to the information processing systems,  
91 fundamental differences exist in the cellular lipids of Archaea and Bacteria. Bacterial  
92 lipids are generally comprised of fatty acids linked to glycerol by ester bonds. The  
93 stereochemistry of the glycerol moiety is the 1,2-*sn*-configuration. Archaeal lipids are

94 comprised of isoprenoid side chains linked to glycerol by ether bonds, where the glycerol  
95 has the 2,3-*sn*-configuration (25, 50). The isoprenoid side chains may contain linear or  
96 cyclopentane units. While these lipids commonly form bilayers as in Bacteria, some  
97 archaeal membranes are monolayers containing glycerol tetraethers that span the  
98 membrane.

99         Few other features are as distinctive of either the Archaea or Bacteria. For  
100 instance, the presence of peptidoglycan in the cell wall was one of the original  
101 characteristics used to define the procaryotes (47). It has since been recognized that the  
102 cell walls of both Bacteria and Archaea are very diverse. Both groups contain wall-less  
103 organisms, such as *Mycoplasma* within the Bacteria and *Thermoplasma* within the  
104 Archaea. Most of the described bacterial cell walls contain a peptidoglycan composed of  
105 muramic acid. Although a few Archaea possess a cell wall composed of peptidoglycan,  
106 muramic acid is absent, and the polymer differs profoundly from bacterial peptidoglycan  
107 (29). Instead, archaeal cell walls are most often comprised solely of S-layers (26). These  
108 are monolayers of a single protein that provide structural integrity and shape to the cell.  
109 S-layers are components of many bacterial cell walls as well, but they only rarely serve as  
110 the sole wall structure as in Archaea.

111         Both groups are also metabolically diverse, and their members have adapted to a  
112 wide range of physiological conditions and life styles (27). They share many of the  
113 major types of transporters for the common organic and inorganic nutrients (37). For  
114 sugar catabolism, variations of the Embden-Meyerhof as well as Entner-Doudoroff  
115 pathways have been described in both archaeal and bacterial heterotrophs (5). While  
116 chlorophyll-based photosynthesis is unknown within the Archaea, both Archaea and

117 Bacteria possess the retinal-based bacteriorhodopsin systems (14). Similarly, the ability  
118 to utilize inorganic substrates for lithotrophic growth, such as H<sub>2</sub>, reduced sulfur  
119 compounds or ammonium, is shared by members of both lineages. The diversity of  
120 anabolic pathways is comparable. While only Bacteria are known to utilize the Calvin  
121 cycle, archaeal as well as bacterial autotrophs use the Ljungdahl-Wood, reverse TCA or  
122 hydroxypropionate pathways of CO<sub>2</sub> fixation (3). Interestingly, while some Archaea  
123 possess ribulose biphosphate carboxylase/oxygenase, the key enzyme of the Calvin  
124 cycle, this enzyme is involved in AMP metabolism and not autotrophic CO<sub>2</sub> fixation (40,  
125 52). Complex variations of intermediary metabolism also occur in both lineages (5).  
126 Many major pathways of monomer biosynthesis are the same in both groups, and some  
127 common variations, such as the citramalate pathway of isoleucine biosynthesis, are also  
128 found in both lineages (10).

129         Thus, in contrast to the genes for the information processing systems, there are no  
130 clear distinctions between archaeal and bacterial metabolic genes (51). As an example,  
131 the genes of the tryptophan biosynthetic operon are widely distributed in both groups  
132 (57). While the genes within each domain are generally more similar to the genes from  
133 the same domain, they are clearly homologous across both domains. There are also no  
134 special features that are characteristic of either domain. Instead, the genes are distributed  
135 according to the physiological adaptations of the specific organisms and complex  
136 histories of gene fusions, insertions, horizontal transfers and other evolutionary events.  
137 There are a few exceptions. Chlorophyll-based photosynthesis appears to be entirely  
138 bacterial and eucaryotic. Methanogenesis appears to be limited to the Archaea.

139           Genomic comparisons are consistent with these conclusions. Within the Archaea,  
140 7538 Clusters of Orthologous Groups of proteins or arCOGs were identified (32). Of  
141 these, 53 % possess a high affinity to bacterial genes, and 42 % appear to be uniquely  
142 archaeal. A small number of arCOGs are common to all archaeal genomes. This “core”  
143 of 166 arCOGs largely includes genes which encode information processing functions.  
144 Of these arCOGs, 77 % possess high similarities to eucaryotic genes. Archaeal genomes  
145 also possess a much larger “shell” of about 2200 arCOGs which are widely distributed  
146 throughout archaeal genomes but are not universal. Many of these genes encode  
147 metabolic functions and are largely shared with Bacteria. The conserved core  
148 distinguishes the Archaea from the Bacteria, but the shell encodes the metabolic features  
149 shared among the prokaryotes.

150           In summary, examination of the distinct and shared characters between the  
151 Archaea and Bacteria provide compelling evidence for two domains. However, the  
152 nature of the evolutionary processes that led to their formation is much less clear (7).  
153 Because of the complexity of the shared features, a model that encompasses only  
154 “vertical” evolution would suggest that the common ancestor was a sophisticated  
155 organism capable of accurate translation and transcription and possessing the metabolic  
156 capability of making all the small molecules in the cell, including complex vitamins such  
157 as cobamides (21). However, the physiological characters shared by both lineages  
158 exceed those found in any single modern organism. For instance, multiple pathways of  
159 glycolysis, autotrophy and respiration are common to both the Archaea and the Bacteria.  
160 This observation suggests that the modern domains are the products of a more complex  
161 evolutionary history which must have included horizontal gene transfers and gene

162 creations and losses as common events (7). This conclusion is supported by the  
163 observation of fairly recent horizontal gene transfers between organisms from each  
164 domain (35). Remarkably, in at least one case, the number of genes involved was  
165 comparable to the number acquired by the ancestor of the eucaryotes during  
166 endosymbiosis (6). Although the consequences may not have been as profound for  
167 biology, this massive horizontal gene transfer illustrates the potential scale of this process  
168 in procaryotic evolution.

169         **Distribution of procaryotes.** Procaryotes are found nearly everywhere in the  
170 modern world, and their presence defines the biosphere (54). They have been detected at  
171 altitudes of 77 km in the atmosphere and depths of 2 km in the subsurface. The soil,  
172 water, sea ice, leaves and roots of trees, guts of invertebrate and vertebrate animals, and  
173 subsurface aquifers are all fully colonized by highly specialized populations of  
174 procaryotes. The number of individual cells is probably on the order of  $5 \times 10^{30}$ , and  
175 their biomass is comparable to that of plants (54).

176         Their abundance enables them to play key roles in the geochemical cycles that  
177 process the major elements of life, including C, N and S. For instance, except for the  
178 noble gases procaryotes contribute to the production of all of the abundant gases within  
179 the earth's atmosphere. For some, such as methane and nitrous oxide, procaryotes  
180 represent the major sources. Likewise, for some of the cycles, key steps are catalyzed  
181 nearly exclusively by procaryotes. Within the nitrogen cycle, biological nitrogen  
182 fixation, denitrification and nitrification are exclusively procaryotic processes. Within  
183 the sulfur cycle, dissimilatory sulfate reduction and anaerobic sulfide oxidation are  
184 exclusively procaryotic.

185           **Diversity of procaryotes.** Microbiologists have long been impressed with the  
186 extreme diversity of procaryotes (47). For instance, prokaryotes in the order  
187 *Planctomycetales* possess true intracytoplasmic membranes, which is one of the defining  
188 characteristics of eucaryotes (30). Within the genus *Gemmata*, these membranes  
189 surround the nucleoid. This structure superficially resembles that of the eukaryotic  
190 nucleus, but the presence of ribosomes inside the compartment is a fundamental  
191 difference. Procaryotes also possess numerous fundamentally different strategies for  
192 motility, including flagellation, gliding, twitching and gas vesicles. Remarkably, even  
193 archaeal and bacterial flagella are not homologous, demonstrating that microorganisms  
194 have acquired similar mechanisms by different means (20). Similar diversity is found in  
195 many other features, including respiration, photosynthesis, cell structure and cell division.  
196 Recently, it has been possible to investigate procaryotic diversity quantitatively. For  
197 instance, surveys of procaryotic 16S rRNA genes in environmental samples have detected  
198 greater than 50 bacterial ‘phyla’, of which only half have cultivated representatives (19,  
199 38). Procaryotic phyla represent the deepest classification within either the Bacteria or  
200 Archaea. Molecular clocks and correlations with the biogeochemical record indicate that  
201 these phyla probably formed greater than 2.5 billion years ago (2, 45). The antiquity of  
202 these lineages is consistent with their enormous diversity. Moreover, the diversification  
203 of the procaryotes preceded the formation of the major lineages of eucaryotes.

204           Given the diversity of ancient groups, it is not surprising that the number of  
205 modern groups is enormous. A procaryotic species is much deeper than common in  
206 eucaryotic biology and includes strains with >70 % DNA:DNA hybridization and a  
207 change in the melting temperature ( $T_m$ ) of the DNA hybrids of <5 °C (53). At present,

208 there are no certain estimates for the total number of procaryotic species on earth. Within  
209 soil, which contains a relatively diverse population, various methods have detected  $10^3$ -  
210  $10^4$  different molecular species or operational taxonomic units (OTUs) per sample (39,  
211 49). Theoretical estimates suggest that soil could contain well over  $10^6$  OTUs (4).  
212 Similar observations have been made in the deep sea (46). In the most extensive study to  
213 date, partial sequencing of 900,000 16S rRNA procaryotic genes from two deep sea sites  
214 encountered 36,087 unique sequences representing 20,468 OTUs (18). The OTUs  
215 detected in these experiments are defined at 97% sequence similarity of the 16S rRNA  
216 and are deeper taxonomic groups than a conventional procaryotic species as defined  
217 above (23). Importantly, only a small fraction of the total number of species known to  
218 exist have ever been characterized.

219       **Classification of procaryotes.** For more than twenty years, a large community of  
220 microbiologists have sought to elucidate a phylogenetic classification of procaryotic life  
221 (12, 15, 31). A major goal of this effort has been to produce a classification that  
222 summarizes our understanding of procaryotes and provides an explanatory, predictive  
223 and conceptual framework for further investigations. Even though the procaryotes are  
224 not monophyletic and the evolutionary processes they have experienced are extremely  
225 complex (34), this classification strategy remains useful, and knowledge of the  
226 evolutionary processes which formed modern organisms provides a great deal of insight  
227 into their biological properties. However, phylogenetic classifications must extend  
228 beyond the simple recognition of monophyletic groups. It must recognize the wide  
229 variety of evolutionary processes known to occur and the realization that not all  
230 evolutionary processes have the same biological consequences. Thus, one important goal

231 of classification is to distinguish groups of organisms which share transformative  
232 evolutionary events.

233         In the case of the procaryote:eucaryote dichotomy, the evolution of the eucaryotes  
234 was such a transformative event. Even though the origin of the eucaryotes is not certain,  
235 the consequences were profound enough to produce an organism fundamentally different  
236 from those not descendent from this event (11). It could be argued that the procaryotes  
237 are then defined by a negative, ie. the failure to undergo this transformative event (36).  
238 This criticism neglects the large number of similarities within the procaryotes that are  
239 part of the basis for this classification. The prokaryotic classification could also be  
240 criticized because it is paraphyletic, but this objection is not substantive if the  
241 identification of monophyletic groups is not a major goal of the classification.

242         Phylogenetic analyses provide additional insights by identifying relationships  
243 among organisms that would not otherwise be obvious. This feature is important  
244 because, while the general properties of prokaryotes are sufficient to unite them into a  
245 single group, the diversity is so large that no single phenotypic or functional property is  
246 likely to be universal. However, even groups with exceptional properties can be related to  
247 more conventional procaryotes by identifying the evolutionary processes responsible for  
248 their formation. This principle was established prior to the development of molecular  
249 phylogenetic methods in the classification of the cyanobacteria within the prokaryotes  
250 (47). Although their photosynthetic apparatus and aspects of cellular differentiation are  
251 similar to those of eucaryotes, these microorganisms possess many features typical of  
252 other procaryotes, such as peptidoglycan in their cell walls, the capacity for nitrogen  
253 fixation, and the absence of intracytoplasmic organelles. In fact, this classification has

254 since withstood the test of molecular phylogenetic methods, and the cyanobacterial  
255 genome is distinctively procaryotic (22). Similarly, even though the planctomycetes  
256 *Pirellula* possesses a true intracytoplasmic membrane, most of its genes are bacterial,  
257 consistent with its classification (16). Genome reduction is also a common process  
258 observed in many independent lineages of symbiotic procaryotes that produces highly  
259 distinctive organisms. In spite of these differences, these organisms are reasonably  
260 classified as procaryotes. From this perspective, even if a modern descendent of the  
261 eucaryotes was phenotypically indistinguishable from the procaryotes, it would remain a  
262 eucaryote based upon its phylogeny.

263         A phylogenetic classification that considers many types of evolutionary processes  
264 as well as their biological consequences easily accommodates chimeric organisms that  
265 are very distinctive by creating a new category. Arguably, the eucaryotes are precisely  
266 such a classification. The classification of groups of chimeric organisms where the  
267 consequences are less dramatic is not necessary problematic. However, it requires a  
268 more complete understanding of the horizontal gene transfer and its role in the properties  
269 of the specific organism.

270         **Conclusions.** The last fifty years has produced a wealth of new information that  
271 greatly enriches our understanding of procaryotes. These organisms have proven to be of  
272 enormous abundance and diversity, the product of complex evolutionary processes over  
273 billions of years. They dominated life during the earth prior to the appearance of  
274 eucaryotes. Their antiquity implies that many of the most salient features of modern life  
275 evolved in an entirely procaryotic world, including most of the organizing principles of  
276 the cell; the basic mechanisms of replication, transcription and translation; the major

277 catabolic and anabolic pathways; and the biogeochemical cycles which maintain the  
278 biosphere. While the term procaryote was precisely defined by Stanier and van Niel (47),  
279 it has occasionally been misused, usually to be synonymous with Bacteria. The solution  
280 to this problem is education about its true meaning and not to discard a valuable and  
281 central concept in biology.

282

283 **Acknowledgement:** I am grateful to my colleagues Dave Coleman, Ford Doolittle, Matt  
284 Kane, Anna Karls, Noel Krieg, John Leigh, Jan Mrazek, Mary Ann Moran, Larry  
285 Shimkets, Jim Staley and Juergen Wiegel, whose constructive criticisms greatly  
286 improved this work. Thanks also to Kamlesh Jangid for preparation of the figure.

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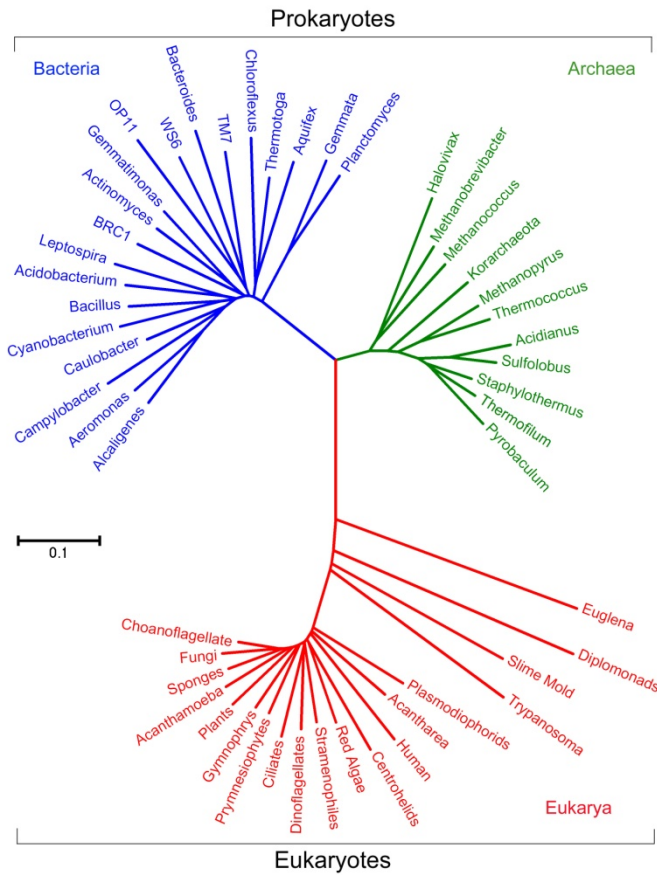
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288 **Figure Legend:**

289 Figure 1. Phylogenetic tree of the 16S rRNA genes in the three domains Archaea,  
290 Bacteria and Eucarya. The procaryotes comprise the archaeal and bacterial domains.  
291 The eucaryotes contain solely the Eucarya domain. This unrooted tree was calculated by  
292 the minimum evolutionary algorithm in the software Mega 4 (48). The tree is drawn to  
293 scale, with branch lengths in evolutionary distance calculated by the maximum composite  
294 likelihood method with units of numbers of base substitutions per site.



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