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Commentary

Archaea: Narrowing the gap between prokaryotes and eukaryotes

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In the last 30 years, our views on the nature of the relationship between prokaryotes and eukaryotes have come full circle. In the 1960s we believed, with Stanier and van Niel, that the line of demarcation between bacteria (including cyanobacteria) and other cellular organisms was Life's "largest and most profound single evolutionary dichotomy" (1). This line was crossed only once, by the prokaryote which first constructed a rudimentary membrane around its genome and cobbled together the first rudimentary cytoskeleton, an entity we used to call the *proto-eukaryote.* Whether we endorsed the then newly resurgent endosymbiont hypothesis for the origins of mitochondria and chloroplasts or envisioned a gentler prokaryote-to-eukaryote (cyanobacterium-to-"uralga") transition, we had to believe that there was once such a bridging organism, whose genome gave rise to the nuclear genome of eukaryotes (2).

For a while, this view seemed to be challenged by the three-kingdom reclassification of living things forced upon us by Woese's exploitation of ribosomal RNA as a tool for phylogenetic reconstruction. Woese showed in 1977 that there are three fundamental cell types and no obvious way to establish which had given rise to which, or whether all three had emerged independently from a common ancestral state so primitive it deserved the special name *progenote* (3). This issue was apparently resolved in 1989 when Iwabe and colleagues (4) and Gogarten and his collaborators (5) provided a root for the universal tree, allowing us once again to begin to reason rigorously about the evolution of major cell types.

This rooting, since confirmed (6), showed that the archaebacteria and eukaryotes are sister groups: the first branch of the tree of life separated the archaebacteria/eukaryote lineage from that leading to eubacteria. In effect, archaebacteria are the closest living prokaryote relatives of the eukaryotes. To mark this distinction, Woese, Kandler, and Wheelis (7) have now given the archaebacteria the formal name Archaea to discourage the common but seldom-voiced opinion that these organisms are really, all things considered, just funny bacteria growing in strange places. A brief but vigorous essentialist debate with Mayr and Margulis ensued, over whether the prokaryote/eukaryote split remains Life's most profound single evolutionary dichotomy, and indeed whether the words *prokaryote* and *eukaryote* retain meaning at all (8–10).

They *are* of course only words. Some of the crucial defining characters of both cell types emphasized by Stanier and van Niel in 1962 would not pass muster today—archaebacteria lack many prokaryotic features which turn out to be strictly eubacterial, while early diverging eukaryotic lineages lack some cytological features we once thought were universal among eukaryotes. But Mayr and Cavalier-Smith insist that we keep the prokaryote/eukaryote dichotomy anyway, that changes occurring in the eukaryotic lineage just after it diverged from the archaebacterial lineage (enclosure of the nucleus, invention of the cytoskeleton, retooling of the chromosomes) were numerous and of an unprecedented and radical kind (8, 11).

This may be sensible, and continuing to refer to the archaebacteria as prokaryotes provides many of us with a certain level of taxonomic stability and comfort. While it is true that the archaebacteria lack certain features originally used to define prokaryotes (such as peptidoglycan), they do share a number of complex features with eubacteria, such as the presence of a single circular chromosome which contains genes arranged in polycistronic operons, often in the same order as their eubacterial counterparts (reviewed in ref. 12). But we must remember that they are prokaryotes of a very different, little-known sort, and are the descendants of an organism closer to the eukaryotes than any known eubacterium.

Even before Woese's definition of the archaebacteria, microbiologists were noticing certain molecular features that can be seen in retrospect to suggest a special relationship between archaebacteria and eukaryotes. Among the first of these were the presence of N-linked glycoproteins, the lack of formylmethionine, shared resistance or sensitivity to various antibiotics, and the presence of tRNA introns (13–15). It is these features (unexpected in prokaryotes as we had come to know them) that arouse the most excitement, and as more and more molecular mechanisms are studied in the archaebacteria, it is becoming apparent that some of these shared similarities run very deep. Several detailed examples exist (16, 17), but the longest-studied and most thoroughly understood is the similarity between eukaryotic and archaebacterial transcription.

Archaebacterial Transcription as the Paradigm Case

This likeness was first recorded in the early 1980s by Wolfram Zillig and colleagues (18), who had discovered archaebacterial DNA-dependent RNA polymerases to be of eukaryote-like complexity. This initial observation has been expanded and confirmed many times over, mostly by work from the Zillig laboratory. That work is summarized, and three more subunits of the *Sulfolobus acidocaldarius* RNA polymerase are described in this issue, by Langer, Hain, Thuriaux, and Zillig (19). Of the 13 sequenced subunits of the *S. acidocaldarius* enzyme, the three largest (B, A′, and A″) are homologs of eubacterial β and β' but still are much closer in sequence to the largest subunits found in each eukaryotic RNA polymerase (I, II, and III). Of the 10 smaller proteins, 6 are homologs of eukaryote-specific subunits shared in some combination among eukaryotic RNA polymerases.

Langer *et al.* (19) also review recent work on the consensus archaebacterial promoter which shows it to be similar in sequence and relative position to its eukaryotic counterpart (20, 21). While details of archaebacterial promoter/polymerase interactions remain imprecisely known, recent studies have uncovered a number of tantalizing eukaryote-like characteristics. It is known for instance that the polymerase itself (like eukaryotic RNA polymerases) is poor at specific promoter recognition (22, 23) and requires at least two other biochemically defined transcription factors to do this *in vitro*, aTFA and aTFB (24). The identity of these factors is becoming clearer. A data base search revealed the presence of an archaebacterial homologue of the eukaryotic basal transcription factor TFIIB in *Pyrococcus* (25). This was followed by the independent discovery, by three separate groups, of the associated TATA-binding protein (TBP) of transcription factor TFIID, the central promoter recognition factor in eukaryotes (26–28). These studies have all been linked together by the demonstration that *Pyrococcus* TBP is able to recognize consensus promoters

and facilitates the binding of TFIIB (27), and that human or yeast TBP can replace aTFB (29). The emerging picture is that promoter recognition and transcription initiation in archaebacteria resemble those processes in eukaryotes: in the words of Langer *et al.*, "the specifying factors are bound to the corresponding promoters and 'hold the door open,' as it were, for the RNA polymerase to attach."

While the actual events of initiation are becoming less of a mystery, no one knows yet what the role of the basal transcription factors is *in vivo*, where promoter recognition is only now being studied (28). There are also a number of other factors which are important in eukaryotes that have not been identified in archaebacteria, and some, like TFIID, that are part of a very large multisubunit complex of which only one component has been identified. The demonstration that two soluble factors are sufficient to promote accurate transcription initiation in archaebacteria suggests that the initiation complex may be somewhat simpler that its eukaryotic counterpart. If this is the case, then the archaebacterial initiation complex could provide some useful details about the nature of the eukaryotic complex, both by clearly defining the roles of the factors in archaebacteria and by identifying which factors are *not* present.

The decades of work on archaebacterial transcription have certainly paid off handsomely. On the other hand, there are numerous processes about which we haven't even begun to *ask* questions: in fact, most of the biochemical criteria that we use to distinguish prokaryotes from eukaryotes are only superficially understood in archaebacteria. In each of these cases a thorough understanding would likely yield as many interesting surprises as transcription has.

Translation: More of the Same?

One example worth considering is the process by which protein synthesis is initiated. Eukaryotic and eubacterial translation initiation involve analogous steps but often differ in how they are accomplished. In eubacteria three initiation factors, IF-1, -2, and -3, are sufficient to direct events in a particular order. First, mRNA is bound to the free small subunit, guided by base pairing between the leader and the 16S rRNA (Shine–Dalgarno and other interactions). Then, formylmethionyl initiator tRNA is imported as part of a ternary complex with IF-2 and GTP. Initiation finally takes place with the binding of the large subunit (reviewed in refs. 30 and 31). In eukaryotes the many common functions are carried out by a different set of factors which number into the dozens, only one of which is homologous (but very distantly) to a eubacterial IF. The order of events and the underlying strategy are also different in eukaryotes, the most obvious difference being the presence of a 5′ cap and the absence of any Shine–Dalgarno interactions. Instead, the ternary complex of eIF-2, GTP, and initiator methionyl-tRNA first binds the dissociated ribosome. This complex then binds to the mRNA through interactions with factors assembled around the cap. The ribosome then scans along the leader, using the tRNA to recognize the first start codon it encounters, and begins protein synthesis (reviewed in ref. 32).

At first, it seemed reasonable to guess that archaebacteria employ a eubacterial-like process of translation initiation. There is no 5′ cap on archaebacterial messages (33), and sequences that could form base pairs with 16S rRNA are found in the leaders of many of them (34). However, as more and more transcription start sites are mapped, it is becoming apparent that a large number of archaebacterial messages have very short leaders, perhaps too short to interact with the 16S rRNA (sometimes they have no leader at all). To account for this, it has been suggested that base pairing may still take place, but does so *downstream* of the start codon, within the coding region itself (35). Unfortunately, there is little direct evidence with which to assess the relevance of these potential interactions.

No archaebacterial translation initiation factor has been identified on the basis of its activity, but there are now several intriguing candidates. The identification of a hypusine-containing protein in several archaebacteria led to the eventual identification of a homologue of eIF-5A (which is distinguished by the presence of this modified amino acid) in *Sulfolobus* (36). *In vitro*, this factor was thought to be involved in the formation of the first peptide bond in eukaryotes, where it is thought to mask the charge of the unformylated methionine (32). This would at first seem to fit the previous observation that archaebacteria, like eukaryotes, also use methionyl-tRNA (14). However, yeast cells depleted of eIF-5A continue to synthesize proteins at an only slightly decreased level, arguing that it is not a general translation factor at all (37).

A homologue of eubacterial IF-2 has also been recognized in *Sulfolobus acidocaldarius* (H. P. Klenk, S. L. Baldauf, P.J.K., W.F.D., and W. Zillig, unpublished work). Although this might be taken to mean that translation initiation in archaebacteria is like that of other prokaryotes, the situation is complicated by the recent identification of a *eukaryotic* homologue of IF-2 (not to be mistaken with eIF-2), which is even more similar in sequence to the *Sulfolobus* open reading frame (ORF). It is not known what this protein does in eukaryotes (38), but if it is part of the translation initiation complex, then it has repeatedly escaped detection, which makes it difficult to decide just what the archaebacterial IF-2 homologue may be doing *in vivo*.

To further complicate this matter, IF-2 is part of a larger family which includes elongation factors EF-1α, EF-Tu, EF-2, and EF-G, as well as the γ subunit of the eukaryotic *analogue* of IF-2, eIF-2γ. A phylogenetic tree of representatives of each of these proteins is shown in Fig. 1, where it can be seen that eIF-2γ is actually only distantly related to IF-2 sequences. Note that all the proteins on the left-hand side of the tree (IF-2, EF-2, and EF-G) recycle GTP by themselves, while the others (eIF-2γ, EF-1α, and EF-Tu) require a guanine nucleotide exchange factor. At some point there was a switch in the proteins used in translation initiation, so that eubacterial initiation complexes include a member of the recycling subfamily (IF-2), whereas the eukaryotic factor arose from within the nonrecycling subfamily (eIF-2γ). The position of eIF-2γ as sister to eubacterial EF-Tu is also strange, and perhaps it implies that eIF-2γ is actually derived from a mitochondrial EF-Tu, but the resolution of this part of the tree is not sufficient to conclude this. In any case, the nature of the factor used by the ancestral initiation complex is unclear; whether it was of the recycling or nonrecycling subfamily is impossible to say, but determining which factors are used by the archaebacteria in protein synthesis initiation would be a great help.

These questions and contradictions on the nature of the initiation complex recently led us to conduct data base homology searches for other archaebacterial ORFs that could code for proteins similar to known IFs. Surprisingly, we found even more. The most compelling similarity is between an ORF upstream of the *Thermoplasma* RNA polymerase operon and eIF-1A (40). The function of eIF-1A (also known as eIF-4C) is to promote dissociation of the ribosomal subunits (it does so by binding free small subunits and really acts by antiassociation), a function which is carried out in eubacteria by IF-1 and 3 (41).

Another as-yet-unidentified ORF in *Sulfolobus acidocaldarius* is similar to GCD1 and GCD6 (H. P. Klenk and P.J.K., unpublished work), two subunits of yeast eIF-2B, the guanine nucleotide exchange factor associated with eIF-2 (42). This example is significantly complicated by the fact that these proteins are also related to a yeast protein, Psa1, that is thought to play a role in protein glycosylation (B. Benton and F. Cross, personal communication), and more distantly to a host of NDP-hexose phosphorylases. The *Sulfolobus* ORF is slightly more similar to Psa1 than to the subunits of eIF-2B, and this being the case, it would be unwise to assign a role in translation initiation to this *Sulfolobus* protein without direct evidence

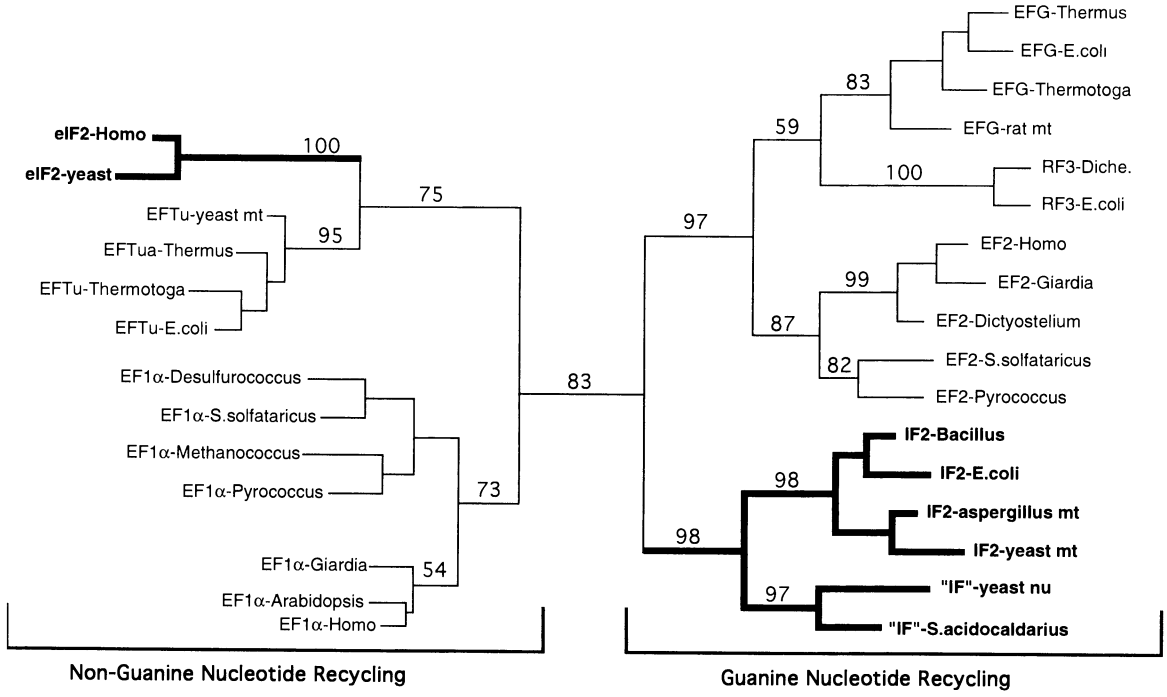

FIG. 1. Phylogenetic tree of selected G proteins involved in translation. Four blocks comprising 111 residues of unambiguously aligned amino acid sequence were subjected to parsimony analysis using PAUP 3.1 (39). A single shortest tree was found in 10 random addition replicates; the numbers represent the percent occurrence of that node in 100 bootstrap iterations. Factors on the right half of the tree are able to recycle GTP, while those on the left require a guanine nucleotide exchange factor. Note that eubacterial IF-2 arises from the former, whereas its eukaryotic counterpart, eIF-2γ, is derived from a nonrecycling factor similar to EF-Tu. mt, Mitochondria; *E. coli, Escherichia coli*; *S. solfataricus, Sulfolobus solfataricus*; *Diche., Dichelobacter nodosus*; nu, nucleus.

(but if it is involved in protein glycosylation it is just as interesting!).

How can we reconcile our current information on translation initiation in archaebacteria? Given the lack of evidence for almost everything, it may not be possible. While the proposed base pairing between 16S rRNA and mRNA may have an important role (34), this has not been directly addressed, and there is growing evidence that the process is not *exactly* like that of *E. coli*. The absence of a 5′ cap also excludes a system entirely like eukaryotes, but the use of nonformylated methionine and the presence of eIFs hint that the mechanism may in certain ways resemble that of eukaryotes. It is also possible that different messages initiate in different ways, some by base-pairing and others by a scanning mechanism that does not require cap recognition. A quick survey of several leaders reveals that most do not contain the triplet AUG before the proper start site (which may lead to premature initiation by a scanning ribosome), but this is also because many are very short. A systematic analysis of more leader sequences could reveal much, but the best possible evidence will come from a detailed study of the initiation process itself.

Other Interesting Processes—A Few Suggestions

Among the other things that distinguish eukaryotes from prokaryotes there are many of which we know nothing at all in archaebacteria, such as translation termination and chromosome segregation, and others for which there are only tantalizing suggestions as to what the analogous process in archaebacteria may be like. Genome replication is a good example; the size and circularity of the archaebacterial chromosome implies that it may be under the same functional constraints as eubacterial chromosomes (reviewed in ref. 12), but that does not necessarily mean that the mechanisms are homologous.

The deeper study of cell division also holds great promise, but it may be complicated by the fact that different kinds of archaebacteria have different means of controlling cell shape (reviewed in ref. 43). It has been suggested that cell division in *Methanocorpusculum* is at least partly a result of irregularities in the crystalline S layer (44). On the other hand, some species do not have rigid S layers, and several appear to have the ability to alter their morphology in a controlled way (45). This has prompted the idea that (at least some) archaebacteria have a cytoskeleton composed of the same components as that of eukaryotes (45, 46). In an interesting correlation, the eukaryotic homologue of hsp60 (cpn60), TCP-1, which is devoted exclusively to folding cytoskeletal proteins (47), is much more similar in sequence (and thus perhaps in function) to its archaebacterial homologues than either is to their eubacterial counterparts.

Another process where progress is being made is motility. Archaebacteria have a rotary motor and rigid flagella, like eubacteria, but the actual flagella are composed of multiple glycoproteins which are more like members of the eubacterial type IV pilin-transport superfamily in both sequence and posttranslational processing than they are to other flagellins (48). The presence of a processed leader peptide implies that flagellins may be transported by the same pathway as other secreted glycoproteins, rather than through the actual core of the filament as in eubacteria (48). The motor itself also has many physical characteristics of a eubacterial rotary motor, but so far none of its components have been identified. The nature of the motor is a point of special interest, as there has been a great deal of work on a sensory reception pathway in halophiles that governs the direction of the motor's rotation. In phototaxis, light-absorbing seven-helix receptors analogous to eukaryotic opsins are coupled to a transducer homologous to the transducers found in eubacterial chemotaxis pathways (reviewed in refs. 49 and 50). In eubacteria this transducer modulates the activity of a histidine kinase, CheA, which was recently characterized in *Halobacterium salinarium*, where it also appears to play a general role in taxis (51). The presence of CheA implies that the switching mechanism in archaebacteria and eubacteria may be of common origin, which would be interesting, as the effects of switching are

quite different. In eubacteria motor rotation leads to either free swimming in a certain direction or random tumbling, while in archaebacteria switching merely changes the direction of swimming (52), a distinction that results in very different demands on the switching mechanism and motor.

Where Do New Processes Come From?

As sequence data accumulate, it is becoming more and more apparent that many proteins, protein families, and molecular processes are common to all life: consider such recent findings as ubiquitin and proteasome-like homologues in bacteria (53, 54), CheA in eukaryotes (55), further evidence of bacteria polyadenylylating mRNA (56), and the growing number of claims for homology based on secondary structures and weak sequence similarities. This latter kind of analysis has provided possible links between tubulin and FtsZ, and between actin, hsp70, and FtsA (57–60), and it is changing the way we think about the evolution of new processes. While cellular processes themselves differ between eukaryotes, eubacteria, and archaebacteria, the components involved in carrying them out seem seldom to have been purposefully built: Jacob's metaphor of evolution as tinkerer is as apt for molecules as for morphology (61).

So at the moment, archaebacteria may give us a better glimpse of the protoeukaryote than any other creatures still alive on Earth. But we must broaden our knowledge base for organisms in all three domains before we can draw any real conclusions about what the ancestor of any domain looked like. For instance, processes such as transcription and translation initiation are poorly understood in the deepest branching eukaryotes (although a few things can now be inferred from what we know about archaebacteria). It would be useful to see whether they use capped mRNA, or a large multisubunit TFIID, or whether they have a fully developed cytoskeleton and chromatin structure. The same principle applies to eubacteria, where a great deal of our understanding centers on a very few organisms. The possibility of overgeneralizing and missing some of the most fascinating relationships between the major divisions of life is real and unfortunate.

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