involves some uncertainty. In essence, one can obtain more data and be more certain as to the accuracy of $t(a)$ for each vessel (*8–10*).

The relaxation time and the variations in carrying out 90° and 180° pulse timings are not significant sources of error because the procedure is iterative for each sequence of data. As a result, there is no linearity requirement in obtaining $t(a)$; instead one has only to observe the time at which the NMR signal of the flowing blood in the vessels stops increasing and remains constant. The error in $t(a)$ is therefore due to lack of sufficient data to measure the crossing points accurately. In the measurements we reported here, we estimate an overall accuracy of about 10 percent for the range of flows encountered.

Each of these measurements was averaged over 512 NMR signals. Each signal took approximately a half-second, so that, because of the averaging, about 4 minutes was given over to obtaining each set of pixels. Of course, all the points relating to 50 msec were obtained as a group, as were all the points relating to 100 msec, and so on. As a consequence, all the flow data for all the veins seen in an NMR image such as the one in Fig. 1 can be obtained in approximately 20 minutes. The computer can be programmed to obtain the flow data for the vessels in the selected slice and provide readouts simultaneously.

It has been proposed that NMR be used to study blood flow in the brain, heart, lungs, kidneys, and vascular system (*8–10*). We believe that the ability to visualize any vessel and quantitatively measure its blood flow will provide a means for monitoring the effectiveness of therapeutic treatments involving the vascular system.

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Sequence of the 16*S* Ribosomal RNA from *Halobacterium volcanii*, an Archaebacterium

Abstract. *The sequence of the 16S ribosomal RNA (rRNA) from the archaebacterium* Halobacterium volcanii *has been determined by DNA sequencing methods. The archaebacterial rRNA is similar to its eubacterial counterpart in secondary structure. Although it is closer in sequence to the eubacterial 16S rRNA than to the eukaryotic 16S-like rRNA, the* H. volcanii *sequence also shows certain points of specific similarity to its eukaryotic counterpart. Since the* H. volcanii *sequence is closer to both the eubacterial and the eukaryotic sequences than these two are to one another, it follows that the archaebacterial sequence resembles their common ancestral sequence more closely than does either of the other two versions.*

There exist three primary phylogenetic groupings: the eukaryotes, the eubacteria, and the archaebacteria (*1–3*). Their comparative analysis will provide us considerable evolutionary perspective. At present, the third of these, the archaebacteria, is poorly understood in molecular terms. For example, of their ribosomes we know only that they are approximately 30*S* and 50*S* in size and that their ribosomal RNA's (rRNA's) are slightly smaller than the corresponding eubacterial rRNA's (*4*). Neither of the large rRNA's nor any of the ribosomal proteins have been fully sequenced. Ribonuclease T_1 catalogs for a number of archaebacterial 16*S* rRNA's have been generated, however (*5*), and partial sequence data for some ribosomal proteins do exist (*6*).

We now report the sequence of a large ribosomal RNA from an archaebacterium, the 16*S* rRNA of *Halobacterium volcanii*. In secondary structure, this rRNA resembles the eubacterial 16*S* rRNA more closely than it resembles the 18*S* rRNA of eukaryotes. Yet in a number of details, it seems remarkably eukaryotic. In primary structure, the archaebacterial 16*S* rRNA is closer to both its eubacterial and eukaryotic counterparts than these two are to one another.

Figure 1 shows the sequence of the *H. volcanii* 16*S* rRNA aligned with its counterparts from *Escherichia coli* and *Xenopus laevis*. The DNA sequence is completely consistent with the catalogs of oligonucleotides produced by digestion of the corresponding rRNA by ribonuclease T_1 (complete catalog, covering 47 percent of the sequence as pentamers or larger), by pancreatic ribonuclease (incomplete catalog), and by ribonuclease U_2 acting on RNA in which the G residues were blocked with glyoxal (incomplete catalog) (*7*). This indicates that no insertions of significant size occur in the gene. The RNA catalogs also permit identification of the functional termini of the 16*S* rRNA gene and placement of post-transcriptionally modified nucleotides.

The *H. volcanii* sequence, consisting of 1472 residues, is significantly shorter than its *E. coli* counterpart, which has 1542 residues (*8*). When the two sequences are optimally aligned (*9*), 59 percent of the positions in the *H. volcanii* sequence are identical to their counterparts in *E. coli* (*8*). This compares to 75 percent homology between *E. coli* 16*S* rRNA and that of the *Zea mays* chloroplast (*10*); these two rRNA's seem to represent the phylogenetic extremes within the eubacteria (*2*). The *H. volcanii* and *E. coli* sequences are very alike in secondary structure as well (see Fig. 2). Of the 50 odd secondary structural elements recognized in the *E. coli* sequence (*9, 11*), only three are absent in the *H. volcanii* version and an additional three or four are present in a somewhat altered form. The rest are virtually identical in the two cases; *H. volcanii* may have one or two helices not seen in its *E. coli* counterpart, but comparative proof for these is, at present, lacking. Furthermore, in all but a few of the homologous helical elements, the *H. volcanii* and *E. coli* versions differ in composition by replacements of at least two base pairs, providing additional support for the helices originally proposed on the basis of comparative studies within the eubacteria alone (*11*).

The degree of secondary structural resemblance between the two bacterial 16*S* rRNA's is greater than either shows with the eukaryotic 18*S* rRNA (*Xenopus laevis*) (*9, 12*). To understand how the three rRNA's are related, however, it is necessary to compare their sequences in

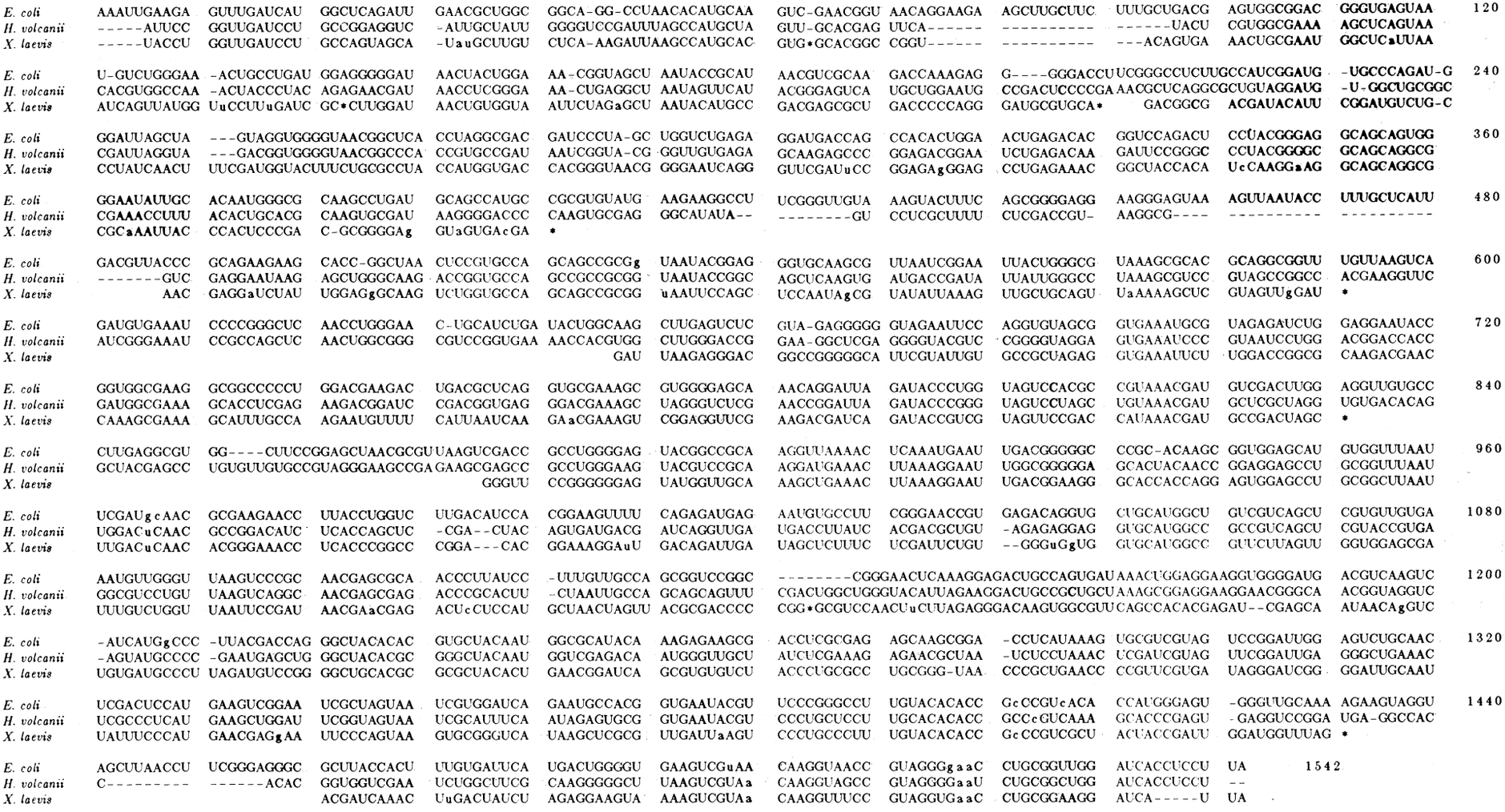

Fig. 1. Sequence of the 16*S* ribosomal RNA from *H. volcanii*. The sequence has been determined completely by DNA sequencing (*25*). Partial RNA sequencing, by oligonucleotide cataloging, has determined the 5′ and 3′ termini, located the modified nucleosides, and in part checked the DNA sequence (for accuracy and insertions) (*7*). Modified residues are shown in lowercase but are not otherwise identified. The *H. volcanii* sequence has been aligned (*9*) with both the *E. coli* (*8, 26*) and *X. laevis* (*12*) 16*S*-like rRNA's (*26*). Portions of the *X. laevis* sequence that are nonhomologous have been deleted; an asterisk denotes the start of a deleted portion. The *H. volcanii* sequence comprises 1472 residues, but the numbering in the figure is that of the *E. coli* sequence (*8, 9*).

detail in regions of reasonably homologous secondary structure; about 30 such regions can be recognized (*9*). This comparison [given more extensively in (*9*)] reveals a number of interesting points.

1) Sequence conservation among the three rRNA's is decidedly greater in nonpaired regions than in paired ones—62 percent of residues as opposed to 33 percent; nonpaired regions also have a lower fraction of positions in which all three sequences differ from one another—7 percent as opposed to 15 percent.

2) Even when regions of clearly nonhomologous secondary structure are eliminated from consideration, the two bacterial versions of the rRNA sequence remain closest to one another. This bias is slight in double-stranded regions but pronounced in the single-stranded ones.

3) The eukaryotic sequence is not equidistant from the two bacterial sequences. It is decidedly closer to the archaebacterial than to the eubacterial one, particularly in the double-stranded regions.

4) Although most regions show the highest degree of homology between the two bacterial sequences, there are at least six regions (*9*) in which the archaebacterial-eukaryotic similarity is greatest but none in which the eukaryotic-eubacterial similarity is greatest.

Other examples of specific details in which the archaebacterial and the eukaryotic sequences resemble one another are the following (*13*). (i) All eukaryotic and archaebacterial sequences begin at position 6 (*14*). (ii) The characteristic invariant eubacterial residues C_{47}, C_{912}, G_{966}, U_{1381}, and C_{1384} are replaced by A_{47}, U_{912}, U_{966}, C_{1381}, and U_{1384} in all archaebacterial and eukaryotic catalogs. (iii) The bulge loop in the universal helix 500–517/534–545 (*9*) contains six bases and begins at position 505 in eubacteria; in the other two groups, it appears to contain seven bases and begins at position 506. (iv) The invariant eubacterial sequence $GCACA_{936}$ in the other two groups contains a pyrimidine insertion near position 933.

The fact that the archaebacterial sequence (in strictly comparable regions) is closer to both of the other two sequences than they are to one another will produce an unrooted phylogenetic tree in which the archaebacterial (*H. volcanii*) branch is the shortest (the eukaryotic branch is the longest). Although the root of this tree cannot be determined, the data demand that the archaebacterial version be the closest of the three to the ancestral version common to all.

The eukaryotic version of the molecule lacks a number of the secondary structural elements common to the two bacterial versions. Moreover, the eukaryotic versions of many of the secondary structural elements common to all three are less regular than are their bacterial counterparts; for example, more noncanonical base "pairs" are found in the eukaryotic helices, and bulged residues within helices are more frequent (*9*). A high degree of irregularity in secondary structure (and uniqueness in sequence) is also characteristic of mitochondrial rRNA's (*9, 15*); these have been shown to be degenerate forms of eubacterial rRNA's (*3, 16*). If we attribute the origin of the eukaryotic rRNA idiosyncrasies to a similar cause, then the eukaryotic rRNA structure is a derived version of a common bacterial rRNA structure, branching from one or the other of the bacterial lines (or from their point of common origin). However, we have no grounds at present for totally dismissing the idea that the irregularities in eukaryotic rRNA represent a more primitive condition and that the eukaryotic branching from the common ancestral stem may have occurred before the split between the two bacterial lines.

The present analysis provides far more comparative sequence information than was available when the existence of archaebacteria was first deduced (*1*). However, the original conclusion that archaebacteria, eubacteria, and eukaryotes are three primary classes of living systems is not altered. This conclusion no longer rests solely on comparative sequence analysis of a single molecular species (16*S* rRNA). Various studies reveal the uniqueness of the archaebacterial phenotype. (i) Their transfer RNA's (tRNA's) contain no ribothymidine at position 54 in the molecule; rather position 54 is often occupied by $m^1\psi$ (*17*). (ii) Their cell walls are of unique compositions (*18*). (iii) Their lipids are ether-linked, and the side chains are branched (phytanyl) (*19*). (iv) Their RNA polymerases have unique subunit structures (*20*).

There is a notion among some biologists that archaebacteria are specifically related to the ancestral eukaryotic cell, the so-called "urkaryote," which hosted

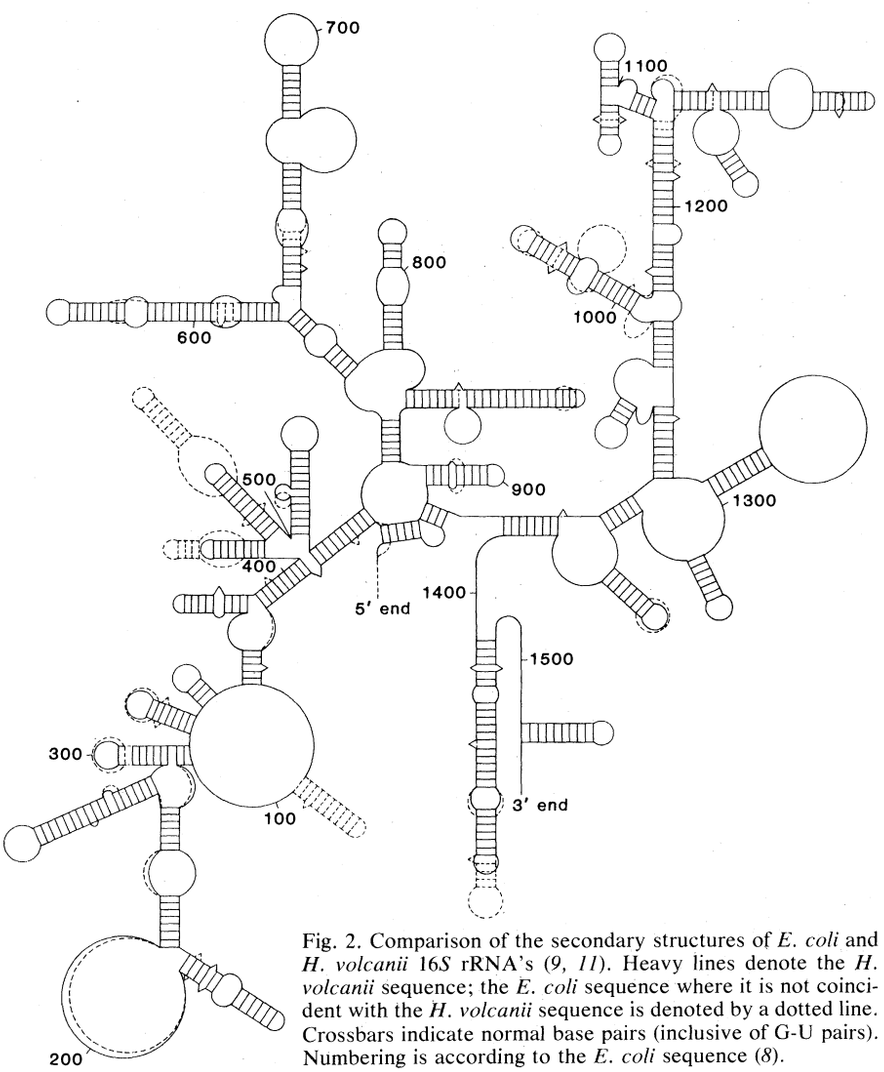

Fig. 2. Comparison of the secondary structures of *E. coli* and *H. volcanii* 16*S* rRNA's (*9, 11*). Heavy lines denote the *H. volcanii* sequence; the *E. coli* sequence where it is not coincident with the *H. volcanii* sequence is denoted by a dotted line. Crossbars indicate normal base pairs (inclusive of G-U pairs). Numbering is according to the *E. coli* sequence (*8*).

the endosymbionts that would become organelles (*3, 21*). This suggestion stems from the growing number of specific resemblances between archaebacteria and eukaryotes. For example, (i) the adenosine diphosphate ribosylation of archaebacterial elongation factor by diptheria toxin (*22*), (ii) the close resemblance between the archaebacterial and eukaryotic versions of the sequence of one or more of the (large subunit) ribosomal proteins (*6*), (iii) the findings that archaebacterial tRNA's may be more readily aminoacylated by eukaryotic than by eubacterial synthetases and that protein synthesis in archaebacteria starts with Met-tRNA, not F-Met–tRNA (*23*), and (iv) the finding that an immunological cross-reaction exists between archaebacterial and eukaryotic RNA polymerases (*24*). It is apparent that the matter of the earliest phylogenetic branchings is far from settled and that the origin of the eukaryotic cell is a deeper and therefore more interesting problem than it was once thought to be.

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5-Hydroxytryptophan Elevates Serum Melatonin

Abstract. *Daytime administration of 5-hydroxytryptophan to sheep elevated serum melatonin more than sevenfold within 2 hours. This suggests that administration of 5-hydroxytryptophan could be used as the basis of a clinical test of pineal function and that melatonin might mediate some clinical effects of 5-hydroxytryptophan.*

5-Hydroxytryptophan (5-HTP), which is used in the treatment of depression and myoclonus (*1*), is an intermediate in the synthesis of *N*-acetyl-5-methoxytryptamine (melatonin). The pathway is tryptophan → 5-HTP → 5-hydroxytryptamine (serotonin) → 5-hydroxy-*N*-acetyltryptamine (*N*-acetylserotonin) → melatonin (*2*). Melatonin synthesis by the pineal gland, which is thought to be the major determinant of the concentration of circulating melatonin (*3*), is low during the day and increases at night as a result of neural stimulation of the activity of serotonin *N*-acetyltransferase (*2*). Even though 5-HTP has been found to cause a small increase in rat pineal melatonin (*4*), the role of this precursor in the control of melatonin synthesis and serum melatonin has generally been ignored. We now report that administration of 5-HTP to sheep during the day increases the concentration of serum melatonin to nighttime levels within 2 hours.

Male sheep that had been housed for 1 week in a windowless stall with automatically regulated lighting were intraperitoneally injected with tryptophan (500 mg/kg), 5-HTP (20 or 200 mg/kg), or saline (*5, 6*). Blood samples were obtained at 1-hour intervals beginning immediately before treatment and ending 5 hours after treatment. Serum melatonin was measured by radioimmunoassay (*7, 8*) and serum tryptophan by a fluorometric technique (*9*). Statistical analysis was done with Duncan's multiple-range test (*10*).

A diurnal rhythm was seen in the level of serum melatonin in saline-treated sheep (Figs. 1 and 2) (*7, 11*). The injection of tryptophan elevated serum tryptophan 10- to 15-fold after 1 to 5 hours (*12*); this caused a very small increase in serum melatonin that was significant ($P < 0.05$) only at 2 hours after the injection. A tryptophan-induced increase in serum melatonin was not detected at night (Fig. 1).

In contrast to the weak effect of tryptophan on serum melatonin, 5-HTP had marked effects (Fig. 2). The 5-HTP injection (20 or 200 mg/kg) caused a statistically significant ($P < 0.05$) increase in serum melatonin after 2 to 5 hours. The peak of the increase induced by 5-HTP at 20 mg/kg occurred after 1 to 2 hours and was more than seven times greater than control values. The 200 mg/kg dose produced a larger and more lasting increase: