artificial deletions tested in gene transfer experiments, would remove this putative silencer thereby liberating the promoter and allowing it to operate in an enhancer-independent manner, whereas in the natural situation or in Ig genes used in gene transfer experiments, the silencer is retained and the promoter is dependent upon an enhancer to suppress the silencer.

At present, it is premature to propose any detailed model of the control circuits that might be involved in Ig gene regulation. However, it appears that the Ig enhancer is equally active in cells representing various stages of B cell development[2,3] whereas the Ig promoter is more active in plasma cells than pre-B and B cells[4]. Thus, it would seem that the promoter and its associated element 'sense' the differentiation state of the cell and direct the appropriate level of gene expression. A role for the enhancer (and the putative silencer) is harder to formulate although it is possible that the enhancer responds to other inductive signals, such as those B cell differentiation factors which appear to induce a high rate of Ig secretion[11]. In this context, the putative silencer might act to reduce the expression as soon as the inductive signal ceases.

Note added in proof

Recently Grosschedl and Baltimore (*Cell* 41, 885–897, 1985) have also demonstrated that the tissue-specificity of Ig gene expression is not solely regulated by the enhancer but also the promoter. These workers have also identified an intragenic sequence implicated in this control.

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Archaebacteria coming of age

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In 1977, Carl Woese and George Fox published a proposal concerning cellular evolution which, because of its radical nature, has only recently gained general understanding and acceptance among microbiologists, geneticists and molecular biologists[1,2]. That proposal was, in part, that prokaryotes comprise two distinct phylogenetic lineages of extraordinarily ancient divergence – the *archaebacteria* (all of the methanogens and extremely halophilic bacteria and some sulfur-dependent thermophiles) and the *eubacteria* (everything else in Bergey's manual).

Initially, this distinction was based on extensive comparisons of the sequences of oligonucleotides released by T1 ribonuclease digestion of 16S ribosomal RNAs. The molecular phylogenetic database has now been expanded, to include the complete sequences of several dozen archaebacterial, eubacterial and eukaryotic 16S (18S) rRNAs. The list of additional basic molecular or biochemical characters which define the archaebacteria as a coherent group, no more akin to eubacteria than to eukaryotes, has grown impressively. It includes: (1) 5S rRNAs and tRNAs unique in primary and secondary structure and patterns of modifications; (2) group-specific ribosome morphologies and ribosomal proteins whose sequences are neither eubacterial nor eukaryotic, but sometimes reminiscent of both; (3) sensitivity, *in vitro*, to diphtheria toxin – heretofore thought to ADP-ribosylate only eukaryotic EF-2; (4) large, multi-subunit RNA polymerases which seem in structure and antigenic properties slightly more like their eukaryotic than their eubacterial homologues; (5) the otherwise unknown presence of reverse gyrases, which positively supercoil the DNA of thermophilic strains; (6) intervening sequences in genes for tRNA, rRNA and possibly proteins – see below; (7) cell surface structures and walls of many types, none containing peptidoglycan; (8) membrane lipids containing branched (not straight) side chains in ether (not ester) linkage to glycerol; and (9) a bewildering variety of other metabolic, physiological or ultrastructural curiosities[3,4].

So the archaebacteria deserve molecular genetic attention on their own merits. More than that, even, they deserve attention because in understanding the as yet confusing mixture of eubacteria-like, eukaryotic-like and truly unique molecular characters the archaebacteria exhibit, we will come to understand the last common ancestor of all three 'primary kingdoms', an entity that Woese and Fox, in the second part of their 1977 proposal[1,2], called the *progenote*. The fact that differences between kingdoms involve some fairly basic features of genetic information transfer means, almost certainly, that the process of information transfer itself (mechanisms of replication, transcription and translation) was still undergoing rapid adaptive evolution (towards greater efficiency, accuracy and speed) in the progenote.

These concepts were clear in general from the first international gathering of archaebacterial molecular biologists, in Munich, in 1981 (Ref. 3). The second meeting of this now much larger group was held again in Munich, late this June*. The concepts have grown richer and deeper because of the accumulated molecular details, of which I can describe only a few.

In an opening lecture, Woese presented a new phylogenetic analysis of available 16S (and 18S) rRNA gene sequences. Many phylogenetic divergences within the archaebacteria are very deep; the deepest seems to be that which separates sulfur-dependent thermophilic strains such as *Sulfolobus*, *Thermoproteus* and *Pyrodictium* from the extensively characterized methanogens. (Perhaps surprisingly, given their quite different biochemistry, the halophilic bacteria and the wall-less thermophile *Thermoplasma* appear to have arisen from within the methanogenic group.) Data from ribosomal protein sequencing (Matheson) electron microscopy of ribosomal particles (Stöffler, Lake), antibiotic sensitivity (Böck, Amils) and functional and structural analyses of translation elongation factors (Klink), 5S rRNA (DeWachter, Fox) and

* EMBO Workshop on the Molecular Genetics of Archaebacteria, organized by A. Böck, D. Oesterhelt and W. Zillig.

RNA polymerases (Zillig) all support this deep division within the archaebacteria. There was much discussion of the notion that sulfur-dependent archaebacteria show a specific relationship to eukaryotes, while the methanogenic-halophilic branch is more eubacteria-like. Thus, archaebacteria may be truly 'archae' - eubacteria and eukaryotes having arisen from *within* them. This idea has been around since the first meeting but the only extensive comparative data to which one can assign unarguable numbers, the 16S rRNA sequences, say it isn't likely to be correct. We need more data of the same quality for other macromolecules.

Much of the nuts-and-bolts in Munich had to do with gene structure, which, given the state of molecular biology in the 1980s, means looking for interesting conserved sequences in and around cloned pieces of DNA. Wich and Böck have scrutinized some 19 tRNA genes from *Methanococcus vannielii*; many are clustered, so there may be rather fewer potential promoters in their collection. Nevertheless, a very respectable AT-rich consensus sequence emerges. Consensus sequences were similarly described for tRNA genes in *Halobacterium volcanii* (Daniels) and by Reeve's and Konisky's groups for several methanogen genes which complement *E. coli* auxotrophs. The sequences themselves are dissimilar, however, and the universal 'archae box' has yet to be found. Perhaps promoters are either too functionally differentiated or too rapidly evolving to pin down in this way. Good data on start sites, *in vivo*, are needed. Dennis has defined, by S1 mapping, five initiation sites of differing strengths upstream of the 16S rRNA gene of *H. cutirubrum*. Each lies within a copy of an (imperfectly) repeated sequence block of some complexity, and a transcription system *in vitro* also seems in order. Thomm and Stetter discussed one such, for *Methanococcus* rDNA. So far, they have limited the promoter for rDNA transcription only down to the first 1000 bp, but refinement should be rapid. As for other functional parts of the genes, one can already see eubacterial-like terminators and can conclude, from the work of Klein, Reeve and Konisky and others that methanogens, at least have recognizable Shine-Dalgarno sequences and produce at least some polycistronic messenger RNAs.

Introns in archaebacterial tRNA genes have been known now for two years[5]. Their presence there has some bearing on arguments about the origin, evolution and function of split-gene organization in general, and one needs to know more. Daniels described an *in-vitro* tRNA-intron processing system for *H. volcanii*. Kjems, in Garrett's group, produced what was, for me, the shocker at this meeting. The 23S rRNA gene of the sulfur-dependent thermophile *Desulfurococcus mobilis*, he finds, sports a 622 bp intron, at a position similar to an interruption in *Physarum* 28S, and this intron forms part of a statistically unequivocal open reading frame which extends into the 3′ 23S exon! Even more gratifying, though yet still tentative, was evidence obtained by Yeats, in Zillig's laboratory, for introns in protein-coding genes of *Sulfolobus* phage SSV1.

There is more to genetics than cloning and sequencing, and two more biologically interesting systems should be mentioned here. First there are the highly unstable strains of *Halobacterium*, particularly *halobium*. As reported by Pfeifer, Betlach and Goebel, instability is due to a very large number of very mobile insertion elements, half-a-dozen of which have been fully characterized, often as insertions into or near the bacteriorhodopsin (*bop*) gene, and these have allowed functional dissection of that locus. Frequent detectable mutations such as those in *bop* may represent only a fraction of insertions in *H. halobium*; most elements reside in (and move around within) AT-rich compartments ('islands') of the halobacterial genome, and most but not of all of those are found on otherwise cryptic and highly unstable plasmids. Second, we may now have a useful system for genetic analysis for halobacteria. Mevarech described high-frequency genetic exchange, which is not transformation and not transduction, between mutants of *H. volcanii* mixed together and brought into contact by filtration on nitrocellulose[6]. How this works is not clear, but it is clear that classical genetic mapping will soon be underway.

The evolution of archaebacteriology as a field, from wonder and awe at the antiquity and deep phylogenetic divisions within organisms alive today to the practicalities of modern recombinant DNA technology, is an expected one. Let us hope we do not lose sight of the truly profound evolutionary questions archaebacteria raise, in our efforts to make them experimentally tractable.

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A single protein that binds to enhancers, promoters and replication origins?

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One model of biological regulation holds that proteins in the cell nucleus function together as a network to mediate the extent of gene expression. Although much progress has been made in identifying individual DNA-binding proteins that regulate transcription, such as the 5S RNA factor TFIIIA, the heat-shock transcription factor, and the glucocorticoid receptor protein, little is known about the architecture of the control network as a whole. The total number of different transcription factors in the cell remains uncertain, for example. Moreover it seems likely that some gene-specific factors may work indirectly, establishing altered patterns of chromatin structure, for example, rather than acting on the transcriptional machinery itself.

With these considerations in mind, it is interesting to examine recent work from several laboratories with a site-specific DNA-binding protein that recognizes the symmetric sequence TGGCANNNTGCCA. This protein was detected by Sippel and co-workers as an activity in oviduct nuclear extracts that bound several regions of the chicken chromosomal DNA flanking the lysozyme gene[1]. The binding appears highly site-specific, and about 25 bp are protected from DNAase digestion at each site[2]. Two closely linked sites map within a region of *in-vivo* DNAase hypersensitivity[3], although the others do not.

Whether the sites are required for proper regulation *in vivo* of lysozyme is still not known. Recent findings indicate, however, that what is apparently the same protein binds to several additional biologically significant regions, including the enhancer of BK virus[4], the promoter of mouse mammary tumor virus (MMTV)[4], the replication origin region at the extreme left end of adenoviruses[4,5], and a hypersensitive region in the 5′ flanking DNA of the *myc* gene[5]. The binding studies in each