

New Ways to Study Developmental Genes in Spore-Forming Bacteria

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The differentiation of cells into specialized types raises the following kinds of questions. In what ways are cells induced to differentiate? How are developmental genes regulated as temporal classes? How is gene expression repressed?

CYANOBACTERIA (blue-green algae) are oxygenic photosynthetic prokaryotes, many strains of which fix N_2 and which probably dominated the Earth's biota during the middle Precambrian, 2,500–570 million years ago. On page 419 of this issue, J. W. Golden, S. J. Robinson and R. Haselkorn report that, despite their ancient origin, the N_2 -fixing photosynthetic cyanobacteria possess a molecular complexity that belies their morphological simplicity¹.

Although long regarded as algae, mainly because of their pigment composition and mode of oxygenic photosynthesis, cyanobacteria are probably more akin to prokaryotic bacteria than to eukaryotic chlorophyllous plants, and are seldom still classified as blue-green algae. They are most noticeable in extreme environments,

for example in parts of Antarctica where they may form 'algal peat', in hot desert regions including parts of the Sahel, on bare rock surfaces, in hot-spring regions and in living stromatolites. Cyanobacteria are also important components of the marine phytoplankton and they, or more probably their colourless analogues, are components of the microflora of the hydrothermal vent regions of the Galapagos Rift. In many parts of the world, but particularly in south-east Asia where fertilizer nitrogen is not readily available, cyanobacteria are important providers of biologically fixed nitrogen for the growth of the rice plant^{2,3}.

It is partly because of their unique ability to fix N_2 , while photosynthesizing in the manner of higher plants that the cyanobacteria have recently aroused the

Nitrogen fixation

Differentiating cyanobacteria rearrange their *nif* genes

from William D.P. Stewart

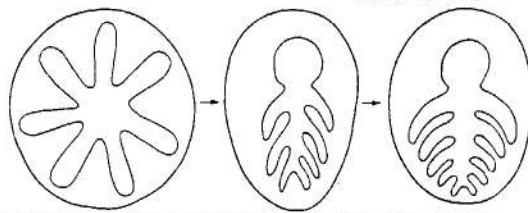
Fig. 2 Evolutionary progression of the metazoan bodyplan from odd-number radial symmetry through "glide reflection" to true bilateral symmetry (M. Fedonkin). See text for details.

A question that arises out of this reappraisal concerns the role of soft-bodied organisms in the evolutionary radiation of the early Cambrian. Many of the more enigmatic fossils seem to represent body plans that are not exhibited by any living creature. The present-day Metazoa (multicellular animals) are dominated by bilaterally symmetrical body plans, often with a degree of segmentation. The coelenterates, with concentric or radial symmetry, today constitute only a small part of the animal kingdom. But M. Fedonkin (USSR Academy of Sciences, Moscow), studying the soft-bodied biota from the Vendian (about 650 Myr) of the White Sea Russian Platform and polar Siberia, concludes that ≈ 70 per cent of animals were then radially or concentrically symmetrical: those with bilateral symmetry are often totally absent.

Some of the more bizarre forms exhibit radial symmetries that today are very rare or non-existent. Thus, an homogeneous group with primary 3-fold symmetry is recognized, and forms with 4- and 7-fold ordering are also common. A particularly important group, according to Fedonkin, is that in which the symmetry remains

radial, but the order (number of arms, gastral cavities, and so forth) actually increases during growth. A possible insight into metazoan evolution comes from fossils such as the late Precambrian *Dickinsonia* which at first sight are bilaterally symmetrical but on closer examination have different numbers of segments on the left- and right-hand sides. Fedonkin terms this phenomenon "glide reflection" and believes that such forms would not be dynamically stable and so evolved to become properly bilateral. He envisages a major evolutionary pattern, recorded in the Vendian rocks, of a gradual change from the dominance of concentric and radial organization, through variable-order radial symmetry and glide reflection, to bilateral segmentation (Fig. 2). A. Seilacher (Universität Tübingen) and others dispute this view, finding no evidence that *Dickinsonia* had a mouth or a gut or any semblance of a metazoan digestive system. Consequently they believe such extraordinary fossils represent not the dawn of metazoans but perhaps a completely different kingdom. □

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attention of molecular biologists. Their study may, in the long term, have relevance to the possibility of introducing the genes responsible for N_2 fixation (the *nif* genes) into the plastids, whether chlorophyllous or not, of higher plants. They have also gained attention as possibly useful models for developmental molecular biologists because many forms occur as simple unbranched filaments with a maximum of three cell types: heterocysts, the sites of N_2 fixation; akinetes, which are perennial; and vegetative cells, from which akinetes and heterocysts develop⁴. Haselkorn and co-workers now add to the scientific attractions of cyanobacteria by showing that they possess a so-far unique capacity to rearrange some of their N_2 -fixing genes, notably two that encode the major components of nitrogenase.

The mechanism that allows the O_2 -sensitive nitrogenase to function in oxygenic cyanobacteria such as *Anabaena* was for long unknown. It was then discovered^{5,6} that the peculiar, empty-looking heterocysts⁷, which occur in most N_2 -fixing cyanobacteria, are the loci of nitrogenase activity in air and in the light, and that by various biochemical modifications, they provide an anaerobic micro-environment in which nitrogenase is synthesized and is functional.

A drawback, until recently⁸, to the detailed genetic analysis of N_2 fixation and heterocyst production in cyanobacteria has been the fact that although mutants of cyanobacteria are readily obtainable, there has existed no good system for the transfer, in the laboratory, of genes into heterocystous cyanobacteria. Thus, genetic analysis by complementation of cyanobacterial mutants has not been possible. An alternative approach, used by Haselkorn and colleagues, is to use the *nif* genes of the enteric bacterium *Klebsiella* as probes for those of *Anabaena*. In *Klebsiella* there are 17 *nif* genes, organized into seven or eight transcriptional units and arranged in a cluster occupying about 23 kilobases of DNA and located near the genes for histidine biosynthesis (see ref. 9). The genes that encode the major nitrogenase components — the iron protein and the iron-molybdenum protein — are *nif K*, *nif D* and *nif H*. The iron protein is composed of two identical subunits, both encoded by *nif H*. The iron-molybdenum protein contains one pair of identical subunits encoded by *nif K* and another by *nif D*. Although the two proteins do not fix N_2 alone, in combination they may do so in the presence of a source of reductant, Mg^{2+} and ATP, and in the absence of O_2 (see ref. 10).

Haselkorn and his colleagues have already shown that, while the *nif K*, *D* and *H* genes are clustered in *Klebsiella* and some other N_2 -fixing organisms, in DNA extracted from filaments of *Anabaena* 7120 the *nif D* and *nif H* genes are contiguous but separated from *nif K* by about 11 kilobases. The Chicago group has

Personal Memo from
JOSHUA LEDERBERG

Dr. Peter Kretschmer
Calanuse

APR 21 1985

Re DNA excision

I think I conveyed some of the excitement that should complicate your perplexity in dealing with programmed DNA loss.

Although I was looking for more material on DNA inversion & transposition in systems like

phase variation in flagellar antigens
E. coli (capsule)
?? sporogenesis + secondary metabolism

This paper on *nif* genes just came up; and it is an exciting precedent.

So is the programmed DNA loss an inducible phenomenon?

Nature 4/4

Science 4/19

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