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THE ORIGIN AND EVOLUTION OF SEX

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Preface

This is the third symposium in the Evolution series conducted as part of the microbiology course at the Marine Biological Laboratory. The first symposium, "The Origin of Life and Evolution," was jointly sponsored by microbiology and physiology courses in 1979. Two years later, the Microbiology and Marine Ecology and the Ecosystems Center held a symposium that compared the first evidences of life on earth with the modern cyanobacter in microbial mats. This workshop had its origins in 1983 when we had agreed that the critical factor in evolution was the development of a sexual mechanism to stabilize the species. Sex is distributed throughout the biological kingdoms, and when it has been analyzed in any detail, is elaborate and involves complex, finely tuned mechanisms. There is no disagreement that from bacteria to man, sex involves DNA replication—and recombination, presumably by the algal mechanisms that have been developed in bacteria.

What is new in the origin of sex? With molecular genetics and gene cloning it is now possible for the first time to analyze in detail chromosome structure and homology. Probes, at the level of genes and chromosome segments, allow us to explore similarities and mechanisms at the genetic level that reveal the developmental programs in sexuality. Understanding the signals—temporal expression or selection pressures—provides a rational basis for posing experimental and meaningful questions about the evolution of sex. We now have a great deal of knowledge based largely on our investigations at the genetic and molecular level. The elegance of understanding of sex in bacteria, yeast and Drosophila are not accidental—but reflect our efforts in genetic studies on these systems. It is clear that sex in *Streptococcus, Tetrahymena, Chlamydomonas,* etc., will be equally complex. With the advent of improved cell manipulations, genetic analysis, and refined molecular probes this complexity can be further revealed.

The evidence is very strong that basic mechanisms, such as protein synthesis and nucleic acid synthesis are highly conserved. One would predict that essential mechanisms for DNA replication, repair, and excision were also used for sex in higher organisms. Many direct tests of these mechanisms—with molecular probes—are now possible. Further, chromosome mobilization is a part of sex in bacteria—and in another form—the

basis of the cassette model for mating type in yeast. One is led to ask, does mobilization of DNA, known to occur in simple eukaryotic cells, play a role in sex expression in higher cells?

Which of the regulatory mechanisms have evolved to control sex? Do they vary more widely than the elements themselves? How widespread is DNA modulation? It is now clear that in bacteria alternate regulatory mechanisms have developed in different species to achieve exactly the same result.

As our understanding of development of sex increases, we have a greater hope for finding or evolving intermediates between eukaryotes and procaryotes in a field that was explored by the late Roger Stanier and summarized in the Symposium on Microbial Mats: Stromatolites.

Progression from unicellular to multicellular eukaryotes is marked by such a diversification of strategies of sex differentiation that it becomes sometimes difficult not to lose the Arianna's thread. One of the most challenging, intractable problems is that of the origin of sex chromosomes. However, it can now be attacked, and is in fact under scrutiny, through the analysis of the conservation and distribution of DNA sequences specific for the sex chromosomes. It is also likely that the molecular approach to sex determination will have important consequences for the understanding of sex abnormalities in humans that appear to be linked to chromosomal abnormalities.

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BACTERIAL CONJUGATION: BEGINNINGS OF SEXUALITY IN PROKARYOTES

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Beginnings of Sexuality in Prokaryotes

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In the last few decades, Schopf [1972], MacGregor [1940], Knoll and Barghoorn [1975], and others found from examination of the oldest known unmetamorphosed sedimentary rocks that there is not only a discontinuity in the carbon-isotope ratios, which is consistent with autotrophic organisms, but also that these rocks contain microscopic organic spheroids, some of which are strikingly reminiscent to dividing cells [for review see Awramik, 1984]. The ancient structures with which some microfossils are associated have been interpreted as stromatolite rocks that are true fossils of microbial mat communities. From these we are left with the conclusion that cellular evolution began as early as 3.5×10^9 years ago. These early undifferentiated fossils are remarkably similar to modern prokaryotes (cyanobacteria). Some are filamentous. Although less clear, the fossil record suggests that it was not until about 10^9 billion years ago, during the late Proterozoic Aeon, that larger flora emerged. Eukaryotic forms appeared in late Proterozoic shales (Knoll and Vidal, 1983; Vidal, 1984) and from the older Austrian dolomites (Schopf, 1978). Presumably, this is in the time during which mitosis and meiosis first arose. Evolutionary biologists have stressed the development of recombination of genetically varied progeny. This could have arisen through fusion not unlike phage infection or the fusion of two haploid cells. Alternatively, algae could have developed a nucleus, chromosomes, and mitotic apparatus. A defect in cytokinesis during mitosis would have led to a homozygous diploid. Variant chromosomes could have arisen leading, without cell division, to chromosomal separation and ultimately to chromosomal reunion. If two or more chromosomes were involved, and cell division occurred after the sep-

aration of homologous chromosomes, gametes would be generated. As pointed out by Bell [1982], this scheme proceeds by known protista steps and puts primary emphasis on division rather than fusion.

In a broader sense, sex is the process whereby DNA is transmitted from one cell to another by mechanisms other than cell division. Because extant bacteria possess recombination mechanisms, this process may have existed as early as 3.5 billion years ago. Genetic exchange can be accomplished by processes such as bacterial conjugation, transformation, and viral recombination. Bacterial matings may be uncommon in nature and restricted to only a few strains as far as we know at the present time. Levin [1981] has argued that even in enteric bacteria, recombination occurs at negligible rates. According to his view, transformation is the exception and sex in bacteria is a laboratory curiosity. Unfortunately, it is unlikely that we will find molecular details of the early stages of the sexual cycle preserved intact in the fossil record. Therefore, an understanding of the origin of sex will have to come from study of contemporary organisms.

Nevertheless, one cannot help but be impressed with the biochemical and molecular similarities between bacteria and eukaryotic cells. Mechanisms exist in bacterial and mammalian cells to carry out related chemical conversions. The key mechanisms of the synthesis of proteins and nucleic acids are remarkably similar. Within bacteria, because of their ability to exchange DNA by transformation, we can readily recognize close relatives in, for example, the *Acinetobacter* family (Juni, 1978). The evolution of pathways, such as the β-keto adipic pathway (Yeh and Ornston, 1981), is readily identified in bacteria. Even genes with similar functions appear to share evolutionary history. In highly conserved genes, such as ribosomal RNA, members of the Archaebacteria can be identified and close relatives readily detected (Woese and Fox, 1977). DNA sequences for essential function, such as cytochrome C (Fitch and Margoliash, 1967) and hemoglobins (Kimura, 1979) have been analyzed to measure the rate of evolution. As our body of knowledge on gene sequences has rapidly expanded, we have gained confidence in concluding that what exists has built upon what went before. The age in which bacteria were the sole replicative life forms on earth were periods of experimentation, in part in unique inhospitable environments. For these bacteria, change was followed by selection in which efficiency was monitored carefully.

It has been assumed frequently [e.g., Haldane, 1954] that sex began very early following the origin of life. Dougherty [1955] proposed, later supported by others [e.g., Smith, 1976], that sex arose as a mechanism to overcome genetic damage. Bernstein [1981; Bernstein et al., 1984a, 1984b] suggested that sexual reproduction originated as a recombinational repair process, first in RNA protocells and later in duplex DNA microorganisms. It is likely that early in evolution there were strong selective pressures to develop mechanisms

for protecting DNA. For this process to evolve, two homologous DNA helices must be present in the same cell. Damage in one could be repaired by information in the other. The process of recombination repair and excision repair have persisted in present-day organisms. During the same evolutionary period, structures and pathways for cell recognition were developed. What is not as yet clear is whether the genes that evolved for these processes were the same ones that evolved for sexuality in eukaryotic cells. Are the genes that evolved for sex in bacteria modified and used for sex in protiston eukaryotes—or in animals or plants? Are there conserved sequences in bacterial sex genes that are recognized in genes regulating sex in higher forms? Alternatively, are separate independent mechanisms evolved for nucleated forms of life? Are the basic mechanisms for DNA replication, repair, and recombination highly conserved genes throughout evolution? With the advent of modern techniques in molecular biology, these questions are answerable.

On the other hand, one could imagine that animals or plants might utilize earlier evolved mechanisms for the exchange of genetic information, for example, cytoskeletal proteins, as the microtubules of the mitotic spindle are used for chromosomal separation during meiosis and mitosis. The elements involved have strong resemblances to structural components in bacteria for motion [Margulis, 1981], cell recognition and response to environmental signals. One could well imagine that structures evolved for another purpose (motility, environmental recognition, etc.) have been incorporated into sexual mechanisms in more complex forms.

Sex represents the most important challenge to the modern theory of evolution. This dilemma was expressed elegantly by Graham Bell in his recent book "The Masterpiece of Nature—the Evolution and Genetics of Sexuality" [1982]. "In the first instance it was long assumed that it (sex) evolved, not only as the result of the normal Darwinian process of natural selection, but through competition between populations or species, an hypothesis elsewhere almost universally discredited. Secondly, attempts to develop a Darwinian theory of sex were hampered by the realization that sexual reproduction usually implies an enormous reduction in fitness because sexual females transmit genetic material only half as fast as asexual females."

A review of the spectrum of mechanism available in bacteria includes the variety of ways in which DNA is organized and transmitted through plasmids and viruses to another bacteria, how such transmitted DNA participates in recombination, and how recipient cells recognize chemical signals that determine mating opportunities. Finally, the nature of genes in bacteria that control the actual mating response will be reviewed.

Our goals are the following

1) To analyze the molecular basis of sex and sexuality in bacteria and

2) To set the foundation for comparison of sex and sexuality in more complex biological forms.

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The Origin and Evolution of Sex, pages 7–12

The Origin of Sex: An Argument

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Sexual reproduction pervades the world of living organisms, yet its origin and evolution remain obscure. The major problem lies in this question: What is the advantage of transmitting only half of one's genes to one's offspring? Various authors have dubbed this the cost of meiosis [Williams, 1975], the cost of sex [Bell, 1982], and the cost of producing males [Maynard-Smith, 1978]. This cost approaches twofold as a maximum, as it only includes the genes that are different in females and males.

Still, it is a paradox. Classifical genetic scholars, such as R. A. Fisher (unpublished), H. J. Muller [1964], Crow and Kimura [1965], Cavalli-Sforza and Bodmer [1971], have postulated that the advantage of sexual reproduction is that it increases the rate of evolution by combining together, far more quickly than could asexual reproduction, new, useful gene mutations. Fisher just stated this point; the others derived equations demonstrating this gene flow. They concluded that the advantage there was a twofold increase in the rate of gene mixing per segregating useful gene with sexual reproduction. One of the parameters in the equations was population size, and population size in a binary event like sex is of considerable importance. Although Crow and Kimura [1965] deemed moderate populations adequate, Cavalli-Sforza and Bodmer [1971] concluded that small populations were best; however, recently, Maynard-Smith [1978] has concluded that populations must be very large i.e., outside the range of reality, for sex to promote the admixture of useful genes. Evidently, we are at a mathematical impasse.

Recent scholarship has used comparative biology as a guide to the value of sex [Williams, 1975; Bell, 1982]. Studies have been made of the distribution of sexual and asexual organisms and the relative kinds of environment they inhabit. Sex is viewed from this perspective as being of immediate value

when it provides genetic diversity in a changing environment. Evolutionary considerations are secondary. Molecular biological considerations have led some to postulate that sex arose because it provides an efficient mechanism for the repair of damage to DNA [Bernstein et al., 1981]. DNA damage could provide a "strong" selective force for chromosome mixing; however, it is necessary that both of the parental chromosomes be damaged. With early sex probably not very efficient, such continuous heavy damage could well drive the population to extinction. Given a similar rate of replication, chromosome damage to haploids would tend to select diploids.

Most of the hypotheses noted above tacitly assume already existing full-blown sexual species. I now present an argument as to how sexuality might have arisen in prokaryotic organisms. A somewhat similar argument was developed by Rose [1983]. I will define sex as any horizontal transmission of DNA. The scenario goes as follows. Consider a protobacterium not too unlike those of the present. It is haploid, has a complement of DNA of about a thousand genes and can double in a reasonably short time. Physical or chemical factors cause the cell to dissolve, releasing and breaking its DNA into pieces (Fig. 1). These pieces can be taken up with some efficiency by nearby organisms. Among the fragments taken up is the one containing the replicator. In the right system, it will be replicated and lead to large numbers, perhaps in turn causing its host to lyse and release these replicons. For them, clearly, there is a strong drive to continue to pass themselves on in a horizontal manner. At that time, perhaps it was easier than currently for macromolecules

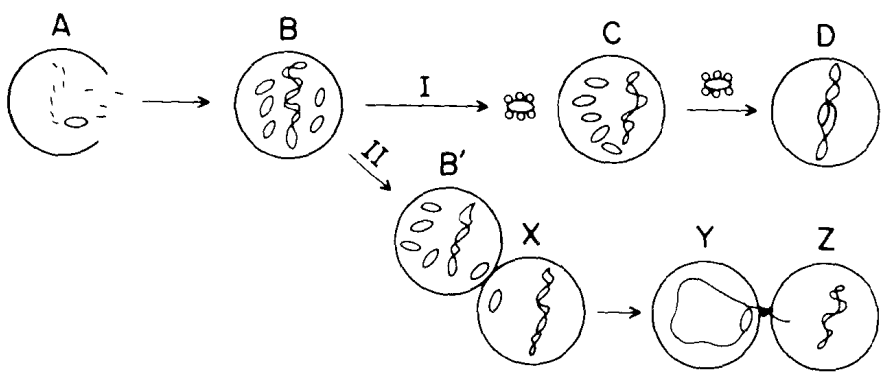

Fig. 1. Scheme for evolution of gene transfer in bacteria. I, Pathway to bacteriophage. II, Pathway to plasmid: A, bacterium lysing; O is replicator segment, --, other genomic fragments; B, transformation of new host and replication of replicator; C, released replicators have coopted bacterial proteins to form protophage; continued cycling leads to phage; D, lysogenization; B′, cycling of plasmid; X, transfer of plasmid, passive at first then involves mechanism; Y, plasmid with transfer genes enters host chromosome mobilizing it for transfer to Z.