ON COMPLEXITY AND SIMPLICITY AS DIFFERENT EVOLUTIONARY STRATEGIES

José L. Martínez
Banco de Células. Biotecnología Microbiana
Centro Nacional de Biotecnología (CSIC)
Campus Universidad Autónoma. Cantoblanco
Ctra. Colmenar Viejo km. 15,500.
28029-Madrid
Spain

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ABSTRACT: Higher eukaryotes and prokaryotes have evolved in quite different ways. The first are complex, thermodynamically expensive organisms, whereas the latter are simple, cheap organisms, with large population sizes. Because of these characteristics, the process of adaptation to environmental changes is different for the two types of organisms. Higher eukaryotes try to maintain the environmental conditions of their cells in a steady state (homeostasis). On the other hand, prokaryotic cells rapidly change their phenotype in response to those modifications. In this way, the response to catastrophic situations is much easier for prokaryotes than for eukaryotes. Actually, bacteria have developed responses to environmental changes that involve the death of most of the population, a situation that higher eukaryotes cannot afford. On the basis of these differences, I propose that complexity and simplicity are not markers of the degree of evolution of an organism, but just different evolutionary strategies. Form this point of view, simple organisms should be an example of jumping evolution, whereas continous walking may be a suitable metaphor for the evolution of complex ones.

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INTRODUCTION

The generation of complexity during evolution has been the subject of a large number of articles, but the reasons underlying this phenomenon are not clearly understood. Complex organisms (multicellular and homeostatic) can modulate the environment, whereas simple ones cannot. On the other hand, the energetic cost of size and complexity is high (Bains, 1987), so the population sizes of complex organisms are much lower than those of simple ones.

This situation has made the strategies for generation of genetic diversity different in the two kinds of organisms. The mechanisms (mutation, genetic recombination) are mainly the same, but the importance of each of them in the acquisition and maintainance of transmisible characters, as well as their own physiological effects, are different.

In this article, recent informations on the mechanisms of generation of diversity in prokaryotes are discussed in comparison to higher eukaryotes. The different roles of programmed cell death (the changes are inheritable in prokaryotes, and not inheritable in higher eukaryotes), and the evolutionary clock of those organisms, are discussed as well.

The comparison is made between prokaryotes (specifically unicellular Eubacteria) and higher eukarytes merely as prototypes of complex and simple organisms, because much less is known of the molecular basis for the generation of genetic diversity in some other groups of organisms, such as archaebacteria, or simpler multicellular eukaryotes (for example sponges), with different levels of complexity. Although they are not the subject of this work, it is relevant to recall that simple unicellular eukaryotes have mechanisms of adaptation which resemble in certain aspects those of prokaryotes more than those of higher eukaryotes. For instance, they have a "common genome" (mobile DNA, see below) that can even be shared with bacteria (Heinemann and Sprague, 1989). They may have extremely fast processes of adaptation to environmental changes and, differing from higher eukaryotes which have somatic cells, any mutation or gene recombination event in any cell is inherited by the progeny and hence may have evolutionary relevance. In this way, it seems that simple organisms (both prokaryotes and eukaryotes) share some strategies of adaptation different from those of complex organisms.

ENVIRONMENTAL CHANGES AND MECHANISMS OF ADAPTATION

Living organisms are able to subsist only in a very narrow window of physicochemical conditions (temperature, pressure, oxygen concentration, etc.). Even when they are living in their ecological niches, they suffer of changes. Sometimes these changes are periodic or otherwise predictable; sometimes they are unpredictable. Whereas higher organisms try to avoid effects of these fluctuations by maintaining homeostasis, and hence the conditions of the medium surrounding each single cell, strategies developed by bacteria are quite different. Two interesting examples of these strategies are the mechanism of switching (van-de-Putte and Goosen, 1992; Plasterk, 1992), applied to programmed changes, and the acquisition of antibiotic resistance, exemplifying non-predictable changes, that we will review in the next section.

The mechanism of switching has been mostly described in relation with bacterial virulence (van-de-Putte and

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Goosen, 1992). Bacterial pathogens need to grow under different conditions during the sequential steps of an infection. For example, an intracellular human parasite will have to live outside the host in different environments, traverse various epithelia, survive in body fluids, pass through the cell membrane, and finally live in the cell. In each of these environments the physicochemical conditions are different, and bacteria need to express different proteins in order to survive.

Two mechanisms of adaptation have so far been described. One is signal transduction, in which bacteria receive signals from the environment by a sensor (usually membrane-located), and modulate the expression of different sets of proteins, depending on the signal received (Miller et al., 1989), changing in this way their phenotype. The other is switching: in the simplest model of switching, the expression of a whole set of genes is controlled by a single protein, but the expression of this protein is not regulated by environmental changes. In a population expressing the regulatory protein, a small fraction of the bacteria (ca. 10^{-5}) have changes at the DNA level that avoid its expression. *Vice versa*, if the bacteria that do not express the protein are the majority, 10^{-5} of them will restore the DNA sequence so that they express the regulator.

The mechanism of this switching in DNA sequence is not completely understood yet. In this case, and in a different way with signal transduction, the adaptation to environmental changes implies genetic modifications that are inherited by the progeny. We can see that during switching, bacterial populations may present an ON/OFF phenotype depending on whether the controller protein is expressed or not. If the population is in an ON state, there is a small fraction of individuals in OFF, so that if a dramatic change in the environment happens, most of the individuals will die, but some of them will still remain and grow to restore the whole population. In this system, bacteria do not sense the environment and change their phenotype. No sensors are involved, and selection is made just by environmental changes.

An example of this mechanism is *Bordetella pertussis*, the etiologic agent of whooping cough. During its life cycle, this organism grows at different temperatures, outside and in different locations inside the host. In each of these situations, different sets of genes are expressed by means of a mechanism of switching (Willems et al., 1990; Stibitz et al., 1989). Actually, the mechanism of adaptation to the different environments involves the death of most of the bacterial population in each of the changes. This mechanism of massive death in response to predictable environmental changes is a viable mechanism of adaptation when the population is extremely large. In higher eukaryotes, the mechanism of programmed cell death is present as well, mostly in response to different stages of development (Vaux, 1993), but the main difference is that in those multicellular organisms, the changes in somatic cells are not in themselves relevant for evolution, and the population of individua cannot afford an adaptation strategy to environmental changes based on the death of most of the individuals. In the case of dramatic changes, the most plausible result would be the disappearance of the species, not its adaptation.

Another general mechanism that allows prokaryotes to afford dramatic environmental changes is gene recombination. All living organisms exhibit gene recombination, but not all recombination events are relevant for evolution, nor do they all have the same function. In higher eukaryotes, only sexual recombination, occurring in very few specialized cells, is involved in the generation of genetic variability, whereas recombination in somatic cells (like antibody generation), which is not transferred to the progeny, is evolutionary irrelevant. In bacteria, two parts of the genome can be distinguished: One is the chromosome, which contains all the indispensable bacterial genes and where recombination events are rare (Smith et al.1991). The other is the so-called "common genome" composed of mobile DNA, such as plasmids, phages and transposons. This part of the genome contains a reservoir of genes that usually are necessary only under special circumstances. In the "common genome", recombination events are frequent, but they differ sexual recombination in higher eukaryotes, which is species specific, in that they are neither species- nor even genus-specific. It has been described that bacterial plasmids are able to mobilize DNA among different kingdoms (Heinemann, 1991; Heinemann and Sprague Jr, 1989) Thus, in higher eukaryotes gene recombination results both in a buffering of mutations, which could be deleterious or produce an undesired gene drifting, and in intraspecific generation of variability. On the other hand, in prokaryotes gene recombination can involve distribution of genes among different genera, genes that are usually dispensable and that can be fixed in the organisms under selective pressure. As a result, higher eukaryotes have a higher constraint in the interchange of genetic material than do prokaryotes. Again, a consequence of this situation is the generation of less variability in the whole population. This fact has two consequences: First, higher eukaryotes preserve their genetic characteristics much better than prokaryotes. Second, prokaryotes can afford fast environmental changes much better than can higher eukaryotes.

EVOLUTIONARY CLOCK

It has been suggested that the evolutionary rate of an organism is a good measurement of its capability to evolve. It has been postulated as well that DNA changes much faster in man than in *E. coli*, due to the presence of introns, transposable elements and sex-related mechanisms of gene recombination. The conclusion from these premises is that the evolutionary clock is faster in man than in *E. coli*.

Two facts oppose this idea: 1) If an organism is completely adapted to its environment, and environmental variation is only less than the length of a generation, its optimal mutation rate should be 0. In this case the organisms with lower mutation rates should be considered as more evolved. 2) It is unclear that the mutation rate is higher in man than in bacteria. For instance, it has been recently published that the mutation rate per genome is similar in different microorganisms (Drake, 1991). On the other hand, although bacteria are able to stably maintain their genetic characteristics for a long time, there is clear evidence showing that the acquisition and establishment of a character is measured in geological time in higher eukaryotes, whereas in prokaryotes the same phenomenon can take just a few years.

When we think of the story of the development of antibiotics, we think of the benefits of their discovery for human beings. But if we think in terms of deaths of individuals belonging to different species, the introduction of antibiotics for the treatment of infectious diseases is probably the most important ecological disaster due to human activities. But what is the response of prokaryotes to the utilization of drugs specifically developed to kill them?: Evolution and fast adaptation to environmental changes. In less than fifty years after the first use of antibiotics, antibiotic resistance has become widely disseminated. In fact, the time for the appearance of resistant strains after introduction of a new antibiotic on the market, is around two years, and in ten years those strains are already disseminated around the world. Dissemination of pathogenic antibiotic-resistant strains is an important health problem (Neu, 1992), but it has ecological and evolutionary implications as well. The genetic bases for antibiotic resistance are: 1) acquisition of mobile DNA encoding for antibiotic resistance ("common genome"; see before), 2) mutation. In both cases, the development of resistance can produce deep changes, both structural and functional, in the bacterial population. An example of this situation is the case of *Streptococcus pneumoniae*. This bacterium was fully susceptible to b-lactam antibiotics until 1975. At this date some resistant strains were isolated, and now they have become widely disseminated, replacing the previously susceptible ones. The targets for the action of b-lactam antibiotics are the "penicillin-binding proteins" (PBPs), which are involved in cell-wall synthesis. In the case of *S. pneumoniae* the resistance to these antibiotics is a consequence of mutations in the genes encoding for the synthesis of several PBPs. But mutations in these proteins imply, not only b-lactam resistance, but important changes in the structure of the cell wall as well. In just a few years we see the evolutionary adaptation of *S. pneumoniae* population to a catastrophic situation (Garcia-Bustos and Tomasz, 1990). Whereas environmental changes (mostly by human activities) produce the loss of thousands of eukaryotic species that are unable to afford those changes, the introduction of toxic substances specially developed to kill prokaryotic organisms does not result in the loss of those species, but rather in their change and successful evolutionary adaptation.

Fast mutational adaptation to environmental changes is a common trait in bacteria. Besides antibiotic resistance, soil bacteria have developed several new metabolic routes to degrade different industrial wastes (van-der-Meer et al.1992). In this case, the mechanisms of adaptation imply the development of new catabolic routes, sometimes using enzymes from different pathways, sometimes developing new enzymes with novel properties. Clearly, mutation at normal rates cannot explain the appearance of those new activities. For instance, it has been calculated that the genes *xyl*E and *nah*H, both involved in degradation of aromatic compounds, should have diverged 50,000 years ago (Harayama et al.1987) whereas, at least in some cases, appearance of new catabolic routes is a matter of months (van-der-Meer et al.1992).

This fast divergence of DNA sequences can be explained by means of three different mechanisms: gene recombination (see before), increase of mutation rate and slipped-strand mispairing.

In a controversial article Cairns et al. (1988) described that lactose-negative *E. coli* strains were able to revert at a higher rate to the prototrophic phenotype when they grew in the presence of lactose than when they grew without the sugar. A hypothesis to explain this phenomenon is the occurrence of mismatches in one or both DNA strands. These mismatches will produce altered mRNAs. If the resultant proteins are selected, the mismatches can be incorporated in both DNA strands. Obviously this phenomenon is dependent on the bacterial mechanisms for DNA repair.

When DNA has repeated elements, another mechanism for increasing mutation rate is slipped-strand mispairing. In model experiments, it has been described that DNA strands can mispair during replication, with the shifted heteroduplex being stabilized by the matching of the repeated units (Levinson and Gutman, 1987). Afterwards, the mispaired bases are repaired using one of the strands as a template.

Again, these mechanisms for increasing mutation rate could be present as well in higher eukaryotes, but whereas every genetic modification is relevant for prokaryotic evolution, this is not true for higher eukaryotes, in which both genetic recombination and the mechanism of reproduction contribute to buffer all genetic changes, making their evolutionary rate much lower.

When bacterial evolution is mimicked in the laboratory, this fast adaptation is produced as well. For example, it has been recently described (Bennet et al.1990) that the adaptation to grow at 42° instead of 37° takes 200 generations for *E. coli*. If we think in a duplication time of one hour, 200 generations is eight days and eight hours. It is difficult to think that a little more than a week can be relevant for the adaptation of a higher eukaryotic organism to a dramatic change such as optimum temperature growth, but it is indeed enough for prokaryotic ones.

CONCLUSIONS

Higher eukaryotes and prokaryotes have developed different strategies for adaptation and hence different strategies for evolution. Whereas continuous walking is the most attractive hypothesis for explaining higher eukaryotes' evolution, prokaryotes are a prototype for jumping evolution. One bacterium can be very similar to another bacterium belonging to the same species, even if they have been isolated in different continents, but this does not mean that bacterial variability is small. They can evolve rapidly in response to environmental changes (for example utilization of antibiotics), and displace the previously established population. This situation agree with Sheldon´s suggestion that "there may be a correlation between *K*-strategists and phyletic gradualism, and *r*-strategists and stasis" (Sheldon, 1990).

Another crucial difference is the way in which natural selection works in both cases. In prokaryotes, the subject for variability is the cell, and it is the subject for selection as well. Any change (mutation, recombination, etc.) occurring in a single cell is relevant for evolution. In higher eukaryotes, the subject for selection is the whole organism, whereas the subject for

variability is only a very small fraction of the organism: the gametes. Therefore in this case only those changes occurring in very few cells are directly relevant for evolution.

Complex organisms have a higher capability than simple ones to control the environment. The price to be paid by this control is that they are energetically expensive and show little flexibility. This implies smaller variability and population size. The consequence of this is that simple and complex organisms respond in different ways to environmental changes. Whereas complex organisms try to avoid these fluctuations by maintaining internal homeostasis, populations of simple organisms always present a full repertoire of alleles ready to be selected by the environment.

Sometimes, it is assumed that conclusions obtained during the study of prokaryotic evolution are equally applicable to the eukaryotic world. Under my point of view this approach is wrong. Although their basic biochemical properties are the same, as well as the forces driving evolution, the different population sizes, organization and manners of reproduction of higher eukaryotes and prokaryotes imply different evolutionary strategies.

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