

Adaptive mutation: shall we survive bacterial genetic skills?

Adaptivna mutacija: bomo preživelii genetske veščine bakterij?

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Abstract. The origin and dynamics of genetic variations is one of the key questions in the modern science that has still not come out with a final answer. Emerging concepts regarding genetic variation have always produced a great controversy because they hold a key to unlock a great mystery of evolution. With such a powerful motivation scientist working in the molecular biology, genetics and biochemistry gathered a vast amount of experimental data showing us that a genome is a dynamic, hierarchically organized and complex integrated system for storing and processing information. Dynamic balance between stability and mutability of DNA nucleotide sequences is essential for a proper functioning of the organism. Beside many DNA repairing proteins and DNA protective mechanisms organisms possess also biochemical systems capable of changing DNA information. One of the most controversial and at the same time the most informative one is a phenomenon called adaptive mutation. We shall review findings concerning the phenomenon of adaptive mutation in prokaryotes and point out an urgent need for the upgrade of the awkward neo-darwinistic view on the origin of the genetic variation.

Keywords: adaptive mutation, inducible mutagenesis, transposable elements, signal transduction network, neo-darwinism

Izvleček. Izvor in dinamika genetskih variacij je eden od ključnih vprašanj moderne znanosti, ki še vedno čaka na dokončen odgovor. Koncepti v zvezi z genetskimi variacijami povzročajo veliko polemik, saj nosijo ključ do skrivnosti evolucije. Molekularni biologi, genetiki in biokemiki, so zavoľjo tako močnega motiva zbrali ogromno količino eksperimentalnih podatkov, ki kažejo, da je genom dinamičen, hierarhično organiziran in kompleksno integriran sistem za shranjevanje in obdelovanje informacij. Dinamično ravnotežje med stabilnostjo in spremenljivostjo DNK zapisa je nujno za pravilno funkcioniranje organizma. Poleg številnih DNK popravljalnih proteinov in DNK varovalnih mehanizmov, imajo organizmi tudi biokemične sisteme, sposobne spreminjanja DNK informacije. Eden najbolj polemičnih in hkrati najbolj poučnih je fenomen imenovan adaptivna mutacija. Najin namen je pregledati dognanja

o adaptivni mutaciji pri prokariontih in pokazati, da je nadgradnja togega neodarvinističnega pogleda na izvor genetskih variacij, nujno potrebna.

Ključne besede: adaptivna mutacija, inducibilna mutageneza, transponibilni elementi, signal prevajalna mreža, neodarvinizem

Introduction

In its essence genetic variation stirred up scientists as far back as the Darwin's era in the 19th century. Despite the fact that Darwin possessed no knowledge about genetics, he suspected that variations between individuals or mutations did not originate only from random events. He thought that there were also adaptive mutations induced by the environment. However, he commented that it was reasonable to treat them as random, as long as we do not know their origin (Darwin 1859).

At the start of the antibiotic era in 1940' the emergence of antibiotic resistant mutants stimulated Luria & Delbrück (1943) to perform a classical experiment, where they exposed bacterial population to a lethal selective pressure of a bacteriophage T1. Phage immediately killed non-resistant cells and only cells with a pre-existing specific mutation could survive the exposure to the phage. A thorough analysis of a number of surviving colonies and their distribution in independent cultures proved the existence of random mutations that arose during the growth with no relation to the selective pressure. Together with the persuasive results from Lederberg & Lederberg (1952) and Cavalli-Sforza & Lederberg (1956) researchers concluded that all mutations arose randomly, prior to or in the absence of the selective pressure. They considered mutations as only a consequence of a non-perfection DNA replication machinery. Genetic variations thus appeared to be totally independent from the needs of the organisms in the environment with natural selection as a final statistical filter to decide which organism will survive. This notion became the central idea of the neo-darvinistic evolutionary biology.

First results that contradicted this well established dogma came already in the 50' when Ryan demonstrated genomic changes without the DNA replication (Ryan & Wainwright 1954, Ryan 1955), but he failed to show that changes follow the applied selective pressure. First indication that the environment can influence the mutation process came much later when Shapiro (1984) used genetically engineered bacterial cells with a mutation that prevented them to use a specific carbon source. By using a non-lethal selective pressure, he observed accumulation of mutants on the plates during the selection.

The turning point in the research on the origin and dynamics of the genetic variation came four years later when Cairns and co-workers (1988) challenged the established biological dogma with an argument that mutations in the direction of phage or antibiotic resistance are not expressed until after the period of growth. Since lethal conditions used killed bacterial cells or at least completely inhibited their growth, classical experiments could not detect and did not exclude the existence of mutations that could arise *after* the selective pressure was applied. The temporal and numerical distribution of surviving mutant colonies clearly demonstrated that during non-lethal and non-mutagenic selective pressure non-growing or slowly growing bacterial cells experience a specific mutation, named *adaptive mutation* that relieves the selective pressure.

The notion that mutations arise in non-dividing stationary cells (Cairns & Foster 1991) was strongly reminiscent of Lamarck's ideas. This, of course, provoked a great upheaval in the scientific community (Lenski & al. 1989, Lenski & Mittler 1993), and it was mainly due to the fact that adaptive mutations arise only after the selective pressure was applied and in the presence of the selective agent. So it seemed that in some way the applied stress directs mutations in a useful way on appropriate sites (Cairns & al. 1988, Foster & Cairns 1992).

After the presentation of such challenging results and thinking, researchers from various countries and different backgrounds performed many new studies and a great deal of experimental data were collected. Improvements in experimental techniques enabled researchers to get a deeper understanding

of the complexity of molecular events taking place during the processes of mutation, which inevitably lead to a general agreement that an awkward classical neo-darwinistic doctrine, claiming that there is no relation between the needs of the organism and its mutability, must be upgraded.

Current findings in adaptive mutation research

Great efforts from many researchers in the last 14 years gave rise to numerous mutational systems, from which some of them became representative and often more explored and understandable. Our intention is to arrange a review of current findings in adaptive mutation research according to such systems and at the end present a discussion on the origin and dynamics of the bacterial genetic variation with pointing out some possible effects of such bacterial genetic variability on the species *Homo sapiens sapiens*.

Cairns system

Escherichia coli cells strain FC40 carry an a+1 frameshift mutation in an F⁺-located lacI-lacZ fusion gene (Cairns & Foster 1991, Foster 1999) and therefore cannot metabolise lactose. After plating on a minimal medium with lactose as the only carbon and energy source, they readily revert to lactose utilization. After many studies and many proposals it turned out that this most popular adaptive mutation assay system, also called Cairns system, is explainable by a standard Darwinian process (Andersson & al. 1998, Hendrickson & al. 2002). The explanation of this phenomenon, called the amplification-mutagenesis model, presupposes that a non-selective growth before plating enables few cells to acquire a simple duplication of the leaky lac mutant allele. After plating, such cells initiate slowly growing clones and with further amplification soon dominate the colony. Eventually a cell of the clone experiences an adaptive mutation that enables it to utilize lactose as a sole carbon and energy source. Selection then favours only stable mutants carrying only the revertant allele; these cells overgrow the clone and begin strongly to predominate in the mature revertant colony.

A recombination between repeated sequences of amplified alleles releases DNA fragments, which are degraded to single DNA strands that induce the SOS system. Induction of the SOS system was confirmed in the *E. coli* strain FC40 (McKenzie & al. 2000). The SOS response induces an error-prone DNA polymerase DinB (Wagner & al. 1999, Wagner & Nohmi 2000, Tang & al. 2000, McKenzie & al. 2001) which leads to extra mismatches that exceed the capacity of the mismatch repair system and therefore a cell experiences a genome-wide mutagenesis known as hypermutation (Hall 1990, Foster 1997, Torkelson & al. 1997, Rosenberg & al. 1998, Rosche & al. 1999, Lombardo & al. 1999, Bull & al. 2000a,b, Cairns 2000, Godoy & Fox 2000a, Godoy & al. 2000b, Foster 2000, Rosenberg 2001, Bridges 2001). Temporary hypermutability is therefore a side effect of the lac operon amplification (Hendrickson & al. 2002).

The amplification-mutagenesis model is totally in tune with the neo-darwinistic view of the origin and dynamics of the genetic variation because here the environment does not direct mutation in specific regions, does not have direct effects on the general mutation rate and mutations do not occur in non-growing stationary cells but in growing subpopulations. But as it will be seen, this model cannot explain other cases of the adaptive mutation in cells under a specific selection pressure.

Adaptive mutation mediated by Mu prophage

Probably the clearest example showing us the incompleteness of the classical neo-darwinistic paradigm is a Mu-mediated adaptive mutation. Bacteriophage Mu is a very notorious mutator phage

that induces mutations in a host genome. Mu element is integrated into the bacterial genome and exists inside the cell in a latent prophage state, as long as the genes controlling its lytic pathway are not expressed. A phage-encoded repressor maintains the control of the expression. In the case of *E. coli* the repressor of the strain used by Shapiro (1984) was temperature sensitive and the Mu prophage was inserted into the *araB* gene. The prophage represents both a translational and a transcriptional block preventing the expression of downstream located genes *lacZ* and *lacY*. When the prophage excises, genes *lacZY* can be fused to an *araB* gene and the cell encodes a hybrid AraB-LacZ protein.

Shapiro (1984) reported that the Mu-mediated formation of *araB-lacZ* fusions occurred when cells were plated on the selective lactose minimal medium with the arabinose as an inducer. Mutants accumulate on the selective media more frequently than during the normal growth. Later it was demonstrated that fusions occurred only in the presence of the lactose (Cairns & al. 1988), but this was subsequently denied. Today it is known that an aerobic starvation could induce the formation of *araB-lacZ* fusions also in the absence of lactose (Mittler & Lenski 1990, Maenhaut-Michel & Shapiro 1994, Foster & Cairns 1994, Sniegowski 1995).

But it turned out that the most staggering point regarding Mu-mediated adaptive mutations was a demonstration that the structures of *araB-lacZ* fusions occurring during aerobic starvation on the glucose or on the lactose-arabinose medium differed from each other (Maenhaut-Michel & Shapiro 1994, Maenhaut-Michel & al. 1997). This means that selective conditions have some influence on the mechanism by which the fusions occur. Through the known complexity of the fusion formation (Shapiro & Leach 1990, Gomez-Gomez & al. 1997, Lamrani & al. 1999) it becomes clear that we are witnessing a multi-step process and not some stochastic individual mutational event. This process enables bacterial cells to control their genetic variation according to the environmental conditions.

Adaptive mutation mediated by insertion sequences

Another example of a mutational event affected by the selective environment represents adaptive mutations in the *ebg* operon mediated by insertion sequences (IS). IS are less than 2,5kb long segments of the DNA that code only elements responsible for their mobility (Mahillon & al. 1998). They are known because of their ability to affect different parts of the genome (Naas 1994 & al., Fedoroff 1999, Shneider & al. 2000) and together with transposons and viruses like Mu constitute a storehouse of mobile DNA elements. IS not only inactivating genes but are also capable of activating the so-called silent or cryptical genes (Reynolds & al. 1981, Hall 1998a, Hall 1999a,b).

E. coli possesses at least four silent systems for the uptake and metabolism of the beta-glucoside sugars (Hall 1998a and references therein). One of them is called the *ebg operon* and it is organized in the same way as the *lac operon*. *Ebg operon* encodes a repressor *EbgR* and a beta-galactosidase *EbgAC* (Hall 1989). If a strain is deleted for the *lacZ*, the *ebgAC* represents the only beta-galactosidase in the cell. The expression of the *ebgAC* is under the control of the repressor *ebgR* and mutations in the *ebgR* gene allow cells to grow on the lactose or related sugars such as lactulose, as a sole source of energy and carbon.

It was shown that during a prolonged starvation on the lactulose 61% of the growth dependent and 80% of adaptive mutations in the *ebgR* gene are mediated by IS (Hall 1999a). 6% of the growth dependent and 39% of adaptive mutations were due to insertions of IS30 in the *ebgR*, so it appears that IS30 transposition may be at least partially directed by some adequate environmental condition (Foster 1999). It was also demonstrated that only adaptive mutations in the *ebgR* gene and not growth dependent mutations are positively regulated by a two-component regulatory system PhoPQ (Hall 1998b).

PhoPQ is a typical two component regulatory system with a regulatory protein in the cytoplasm and a sensory kinase located in the membrane (Stock & al. 2000). Primary signal for the PhoQ sensory

kinase is an extracellular Mg^{2+} (Soncini & al. 1996, Vescovi & al. 1997). After an extracellular signal is sensed by the sensory kinase, an autophosphorylation takes place at the histidine residue of the sensory kinase, thus creating a high-energy phosphoryl group. This group is then transferred to an aspartate residue in the regulatory protein, which induces a conformational change in the regulatory domain. The result is an activation of at least 50 genes in the *E. coli* (Kasahara & al. 1992, Groisman 2001). PhoPQ regulated proteins are therefore directly or indirectly responsible for the adaptive mutation at the *ebgR* repressor gene.

Promoter-creating mutations

An incredible adaptation to a starving condition still unexplainable by the classical neo-darwinistic approach was shown with *Pseudomonas putida* cells carrying a plasmid with a promoterless *pheAB* operon (Kasak & al. 1997) that encodes for the enzyme that decompose phenol. Phenol utilising mutants that accumulated in a starving culture experience base substitutions, deletions and insertions of Tn4652 that created active promoters permitting starving *P.putida* cells to use phenol as a sole source of carbon. Mutants accumulate only in the presence of the phenol and plating stationary phase cells showed higher rate of mutants' accumulation than plating exponential cells.

Another exiting and still unexplainable case of the adaptive mutation is the accumulation of resistant mutants during a non-lethal selective pressure from the antibiotic chloramfenicol (Lioy & al. 2001). During stressful selective conditions sensitive *E. coli* cells carrying a plasmid with a *cam* promoterless gene, encoding for chloramfenicol acetyl transferase enzyme, experience the insertion of the IS10R mobile element into the upstream of the *cam* gene. Mobile element is transposed from the bacterial genome and allowed the bacterial RNA polymerase to efficiently transcript *cam* gene located on the plasmid, which in turn enable cell to survive a non-lethal selective pressure from the antibiotic.

Discussion

Ever since the discovery of the transposable elements in 1950 (McClintock 1984) genome has been viewed as a complex, dynamical and highly organised system for storing and processing information (Berg & Howe 1989, Shapiro 1991, Shapiro 1992, Shapiro 1999a,b, Fedoroff 1999). This means that a bacterial genome is not only a conservative library of triplet codes for cell's constituent elements but also represents a dynamical storage system subject to constant and at least partially regulated changes. After this short but thorough review of the current status in the adaptive mutation research it is obvious that bacterial genetic change can originate from growth dependent random events independently from the environment or from some processes that show sensitivity to specific environmental conditions. The big question is which mode of the mutation generation is more biologically relevant.

To answer that we have to bear in mind the fact that within bacterial genomes coexist highly mutable 'contingency' genes and so called 'housekeeping' genes with low mutation rates (Moxon & al. 1994). This means that not all parts of the bacterial genome are equally mutable and bacterial cells obviously have capacities to control and maintain a balance between stability and mutability of DNA nucleotide sequences. With this in mind it is not surprising that bacterial cells are known to possess many DNA repairing proteins and DNA protecting mechanisms which preserve nucleotide sequences (Radman & al. 1999) and protect organism's needing triplet codes from unpredictable random mutations. However, on the other hand, many times bacterial cells exit stressful conditions only if they acquire specific genetic information. Cells that gain such information sometimes in the past through a random mutational event are not sensing any stress at all. But other sensitive cells that are threatened can

accomplish such a task with *de novo* mutation or with a reorganization of the already existing genetic information that may include a horizontal gene transfer or insertions of transposable elements.

Knowledge gained from the adaptive mutation research demonstrates that during non-lethal stress bacterial cells can experience controlled DNA changes influenced by selective environmental conditions. Bacterial cells must therefore possess some biochemical systems, active only under stress, capable of inducing changes or the reorganisation of genetic information (Shapiro 1991, Shapiro 1992, Shapiro 1997, Radman & al. 1999, Shapiro 1999a,b, Capy 2000). Biochemical systems such as ROSE mutagenesis (Taddei & al. 1995, Taddei & al. 1997), SOS mutagenesis (Fijalkowska & al. 1997) and the adaptive mutation process constitute strategic mechanisms for the genetic variation available to cells during stressful situations. Which cell's response or which inducible biochemical system will predominate depends on many external and internal signals sensed by the cellular signal transduction network. Or in another words, these inducible biochemical systems are regulated by specific controlling mechanisms and show sensitivity to environmental conditions sensed through a complex bacterial signal transduction network (Shapiro 1999a, Massey & al. 1999, Foster 1999).

Conclusions

It may be concluded that both random mutations and controlled genetic changes are present as a constituent elements of the bacterial life cycle. Growth dependent random mutations are unpredictable and bacterial cells are trying to suppress them by numerous repairing strategies. On the other hand, controlled inducible DNA changes are needed during stressful conditions and help many bacterial cells to conquer stress and stay alive. Therefore, we must acknowledge that bacterial cells possess genetic skills that enable a bacterial cell to gain the needed DNA information to exit stress state. We can say that the bacterial genome is evolved to cope with the predictable and also unpredictable challenges that come out from the environment (Caporale 1999).

Last but not least, let us discuss some of the consequences of such bacterial genetic skills on our human lives. Adaptive mutations were demonstrated to be important in a development of antibiotic resistance mutations (Riesenfeld & al 1997, Alonso & al. 1999, Martinez & Baquero 2000, Karunakaran & Davies 2000) and may also provide models for human cancer (Strauss 1992, Hall 1995 Cairns 1998). Needless to say, these two phenomena represent a gigantic problem for modern human society. But at the same time they present a high motivation for researchers to try to understand more deeply and thoroughly the nature of bacterial genetic variation. Therefore it rests on us, researchers, to stay open minded and admit that bacterial cells possess genetic skills that go beyond the ideas of the ordinary neo-darwinian evolutionary doctrine and should not be underestimated.

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