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A Relay Race on the Evolutionary Adaptation Spectrum

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Adaptation is the process in which organisms improve their fitness by changing their phenotype using genetic or non-genetic mechanisms. The adaptation toolbox consists of varied molecular and genetic means that we posit span an almost continuous "adaptation spectrum." Different adaptations are characterized by the time needed for organisms to attain them and by their duration. We suggest that organisms often adapt by progressing the adaptation spectrum, starting with rapidly attained physiological and epigenetic adaptations and culminating with slower long-lasting genetic ones. A tantalizing possibility is that earlier adaptations facilitate realization of later ones.

When challenged by new conditions, organisms adapt by changing their phenotype to improve fitness. The adaptation toolbox consists of varied molecular and genetic means: physiological acclimation, epigenetic changes, structural re-arrangements of the genome, and changes in the DNA sequence. Physiological responses, such as gene expression changes, are often the first to emerge upon environmental changes. Yet, although physiological adaptations may confer selective advantage, they are not actively amplified, memorized, or propagated over many generations. On the next level are epigenetic adaptations, which are distinct from the physiological adaptations, as they can have varying degrees of self-perpetuation over time, and they may occur at the DNA and chromatin, RNA, and even protein. As such, they constitute a molecular "memory." A next level on the spectrum is that of DNA copy-number adaptations, which include segmental DNA duplications/deletions that may range from specific genes to whole chromosomes. These are relatively labile genetic changes, although they do not involve changes in the actual nucleotide sequence of the genome. Lastly, genomic mutations represent the ultimate level of adaptation in which specific changes are stored and inherited relatively faithfully for prolonged periods. The diverse adaptations along the spectrum differ by several important attributes: the time needed for the adaptation to be attained at the individual organism level, the time until the adaptation becomes frequent in the population, the duration through which the adaptation can be sustained beyond the presence of the external selective pressure and when it is relieved, and the faithfulness and accuracy at which the adaptation is propagated across generations (Figure 1). When a challenge persists for longer durations, early adaptations that have been obtained may be subsequently replaced by a more durable adaptation. Indeed, it is often observed that adaptations at the various levels may facilitate one another, e.g., transcription changes can induce chromatin-based modifications (Henikoff and Shilatifard, 2011) and chromosome aneuploidy facilitates mutations in the DNA (Sheltzer et al., 2011). Although adapting organisms need not necessarily move linearly and uni-directionally along this "adaptation spectrum," the effective timescales of the different adaptations may dictate a tendency to move along the spectrum, from the short-lived physiological changes toward the long-lasting genetic ones. For example, a recent study on malaria discovered that *P. falciparum*-acquired drug resistance is a step-wise adaptation process. A non-genetic adaptation to the drug precedes duplications and mutations of the gene that confers the drug resistance (Herman et al., 2014). Along this line, we would like to hypothesize that organisms can perform a "relay race" on the adaptation spectrum.

Below, we discuss adaptations at each level of the spectrum—the context in which they occur and their typical timescales. Further we highlight cases of adaptation that may support the "relay race" notion, as some adaptions happen sequentially, each paving the way for the next to occur.

Physiological Adaptations

When stressed, organisms often acclimate quickly by a series of physiological responses. For example, in the yeast *S. cerevisiae*, a significant portion of the transcriptome changes in response to diverse environmental stressors such as extreme temperature, pH, salinity, and various drugs. These transcriptional plasticity responses are often temporary, as fast relaxation of the response is observed within minutes or hours, even as the stress prevails (Causton et al., 2001; Gasch et al., 2000; Shalem et al., 2008). What is the nature of this response? Genes that are needed to cope with the stress are often induced, e.g., heat-shock chaperones and anti-oxidants enzymes, while genes needed to sustain growth under optimal conditions like ribosomal genes are repressed. The durability of gene expression changes can vary and in some cases can persist across cellular generations. For example, when yeast cells are switched from glucose into galactose, they upregulate the galactose genes (Zacharioudakis et al., 2007). Yet, if switched back into glucose for one generation and then again into galactose, the expression response will be faster than at first encounter with galactose, suggesting a memory of the previous exposure to galactose. Even when grown for up to seven generations away from galactose, cells still "remember" the previous galactose experience.

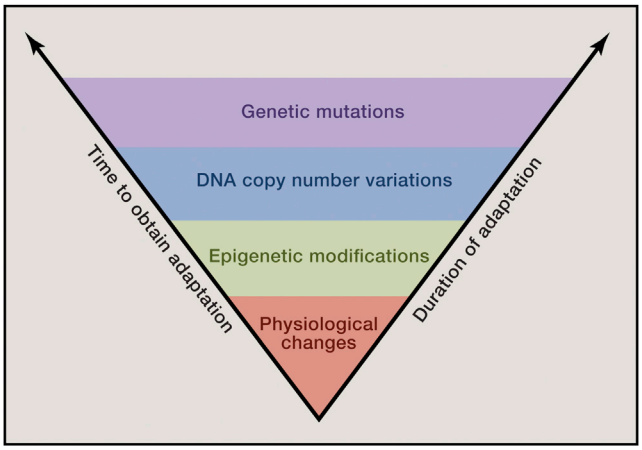


Figure 1. The Different Levels of the Adaptation Spectrum
Two timescales characterize the different adaptation levels: the time needed to acquire the adaptation (left axis) and the time along which the adaptation can be maintained in the absence of the condition that originally required the adaptation. Physiological adaptations consist of changes in current biochemical homeostasis, and therefore their duration depends on the lifetime of those biomolecules that underlie the adaptation, like mRNAs, proteins, etc. Epigenetic modifications typically occur within the same generation that experience the trigger, yet their duration depends on the type of epigenetic mechanism, e.g., DNA methylation, chromatin modification, prions, etc. (reviewed by Rando and Verstrepen, 2007). Genomic duplications include segmental duplications and aneuploidy as a result of chromosomal mis-segregation during cell cycle that result in crude changes in gene expression of the duplicated region. Genetic mutations are functional changes in the coding sequence or regulatory regions of genes that alter their function or expression.

Where and how is this memory stored? It was previously believed that the memory is implemented by nuclear factors that determine rate of transcription re-activation upon re-encounter with galactose (Brickner et al., 2007; Kundu et al., 2007). Yet, a later study clearly showed that the memory is stored in the cytoplasm, in the form of a signaling protein (Zacharioudakis et al., 2007; Ptashne, 2008). It is thus suggested that dilution of the protein in every cell division limits the durability of this memory to seven generations. More recent work on similar "phenotypic memory" showed a memory of up to ten generations when *E. coli* cells were subjected to rapidly alternating carbon sources. This memory mechanism, termed "response memory," appeared to be a hysteretic behavior in which gene expression persists after removal of its external inducer, and this enhances adaptation when environments fluctuate over short timescales (Lambert and Kussell, 2014).

In an environment that changes in a predictive manner, gene expression programs were found to encode "anticipation" of the subsequent environmental changes so that genes are expressed prior to the occurrence of the stimulus that normally activates them (Brunke and Hube, 2014; Mitchell et al., 2009; Tagkopoulos et al., 2008). But what if the challenges are unfamiliar? Response to an unforeseen challenge may require a dedicated strategy. In one study in yeast, a gene that is essential under the applied conditions was placed under a promoter that precludes its expression (Stern et al., 2007). Cells were thus trapped in a situation in which they must express a gene, which they possess, though in an inaccessible regulatory form. After approximately ten generations, a solution appears to have been found, as the population restored the ability to grow. The nature of this solution remains largely unknown. A potentially useful hint appears to be that, when genome-wide transcription was monitored, it was found to be different in each repetition of the experiment, suggesting that the cell's strategy might be to deliberately introduce noise to their expression program such that each cell will gamble on a potentially unique solution. In that respect, it could be appreciated that, like genetic mutations, which are predominantly neutral, gene expression changes might be neutral too (Koonin, 2007). Yet, whether noisy expression is the solution to the unforeseen challenge in this case is still an open question.

Rapid physiological reaction to a challenge is common among cells in the population and appears to be a first line of adaptation, which is mostly based on a hard-wired reaction to stimuli. This reaction is considered adaptive, as it not only improves the fitness under the current occurrence of the challenge, but as mentioned above, it might also improve the ability of the organism to cope with immediately subsequent occurrences of this challenge. In our context, physiological adaptation is defined by a lack of ability to actively perpetuate a memory. Nonetheless, changes in gene expression may serve as a substrate for downstream epigenetic modifications that can prolong their effect.

Epigenetic Adaptations

In this section, we distinguish physiological adaptations from the next modes of adaptation that feature active mechanisms for memory propagation across generations. Despite the controversy over the diverse definitions of epigenetics (Riddihough and Zahn, 2010), it is probably within the consensus that they involve some active mechanisms to perpetuate a memory across (cellular or even organismal) generations. As we will discuss, this epigenetic memory can be implemented at any level of the Central Dogma.

Inheritance by DNA Methylation and Chromatin Modifications

Apart from the nucleotide sequence itself, information on the DNA can be dynamically modified at two prime levels that constitute a major form of "epigenetics." One prime source of epigenetic information is implemented by covalent modification of DNA bases; the other is implemented by histones. DNA methylation of CpG dinucleotide in promoters with CpG islands is generally considered transcriptionally repressive (Cedar and Bergman, 2009). Due to the palindromic nature of CpG di-nucleotides, methylation of the C residue in the parental DNA strain can be easily restored in the new strand after cell division by recognition and methylation of hemi-methylated sites. Yet, the capacity to inherit epigenetic changes across organismal generations, e.g., in animals, is limited since it is *mostly* erased in the early embryo and then re-established in each individual (Smith et al., 2014).

DNA methylation is not the only epigenetic change that occurs on chromatin. Histone modifications, which occur in an impressive diversity of chemical forms and types, are long known to be associated with different states of transcription (Jenuwein and Allis, 2001). The "histone code" hypothesis asserts that some of the many modifications that take place on histones affect, either positively or negatively, transcription levels. However,

the issue is highly controversial (Henikoff and Shilatifard, 2011), as the main evidence for association between a particular chromatin modification and transcription activity level is often correlative rather than causal. Thus, the alternative to the histone code hypothesis is that certain marks on chromatin *result from*, rather than *cause*, a transcriptionally active or repressed state (Henikoff and Shilatifard, 2011). The accumulation of evidence in favor of each of the two directions may suggest a reconciled reality in which transcription state determines certain histone modifications, of which some can, in turn, affect transcription. Assume conservatively that transcription activation of a gene was regulated by a conventional transcription factor and that this change has consequently affected some histone marks in the vicinity of the regulated gene. These and other histone changes may sustain and perpetuate further the initial transcriptional activation. In other words, the mutual effect of transcription activity and histone marks could serve as memory loop with improved self-perpetuation capacity that transmits a purely physiological transcription change into the longer enduring epigenetic level. One demonstration of the effect of chromatin regulation was observed in an experiment that confronted flies with an unfamiliar challenge (a toxin), for which they were armed with a defense mechanism yet without a suitable regulatory program. Upon the first encounter with the toxin, flies had to suppress chromatin remodelers, the Polycomb genes, in order to activate the defense gene. This change appears to have led to the de-repression of developmental regulators in the affected organ (Stern et al., 2012), and some of the developmental alterations were epigenetically inherited by subsequent generations of unchallenged offspring. The possibility that histone marks are transferred across generations remains an open issue (Moazed, 2011). Nonetheless, recent indications from fission yeast show that chromatin marks can be inherited across many cell generations, independently of DNA sequence, DNA methylation, or RNA interference. Thus, histone marks constitute epigenetic information that can be perpetuated long after the removal of the initiating trigger (Audergon et al., 2015; Ragunathan et al., 2014).

RNA Inheritance

RNA can also transmit epigenetic information between generations. In *C. elegans*, dsRNA-mediated silencing has been shown to produce heritable responses (Fire et al., 1998). Recently, it has been further demonstrated that this nematode utilizes heritable RNAi responses to cope with environmental stresses. In one case, RNAi response was shown to be adaptive by silencing an infectious viral genome (Rechavi et al., 2011). In addition to viruses, heritable small RNAs serve to ward off other genomic parasites such as transposons (Ashe et al., 2012; Shirayama et al., 2012). In another case, RNA inheritance enabled memory of an environmental challenge even when no foreign DNA is incorporated: it was shown that RNAi response can be inherited following a developmental arrest caused by starvation (Rechavi et al., 2014). In this context, the effect also increased the longevity of the progeny by targeting genes with a role in nutrition. Importantly, it was found that this induced gene silencing is transmitted in a non-Mendelian manner that is not dependent on a DNA template but, rather, on an RNA-dependent RNA polymerase, which replicates the RNA to a sufficiently high level that overcomes the dilution effect across generations. In addition, small RNAs specifically induce the production of new small RNAs that spread also to nearby sequences (Sapetschnig et al., 2015). Notably, inheritance of small RNAs is dependent on specific factors, which are required for RNAi inheritance, but not for RNAi per se (Buckley et al., 2012).

In summary, RNA has been shown to propagate memory via different types of self-reinforcing epigenetic loops. These diverse classes of non-coding RNAs emerge as key regulators of gene expression typically by modifying chromatin structure and silence transcription (Holoch and Moazed, 2015).

Protein-Based Inheritance

Prions constitute a unique mechanism to perpetuate protein-based phenotypic changes. Unlike most proteins, prions can assume more than one stable conformation, in which the prionic conformation can serve as an auto-catalyst that can convert other conformations to the prion conformation (DeArmond and Prusiner, 2003). Importantly, prions can be acquired from the environment, e.g., through the diet, such as in the case of the Prion Protein Mad Cow Disease, or in response to other environmental changes (Lindquist, 1996). Such is the case of the translation terminator *SUP35* in yeast. In its non-prionic form, this protein serves as a release factor, needed for the proper translation termination of the ribosome at STOP codons. Yet, in its prion version, this protein aggregates and becomes less effective and accurate in terminating translation (Shorter and Lindquist, 2005). The outcome is therefore an extension of the polypeptide beyond the canonical STOP codon in a mechanism that might allow proteins to be extended with some stochasticity and potentially result in a population with enhanced phenotypic diversity (Halfmann et al., 2012). Like the above-mentioned stochastic transcriptome response and DNA methylation, this mechanism too can be activated upon stress (Halfmann and Lindquist, 2010), thus rapidly disseminating non-genetic diversity when diversity might be most needed. Yet, the feature that makes prion-based response truly exciting is its self-perpetuating nature. The autocatalytic tendency of the aggregation form appears to act as an epigenetic memory that perpetuates through generation and generates non-genetic diversity upon which natural selection can act. More recent work on another yeast prion, [GAR+], demonstrated how a prion can become adaptive by allowing cells to utilize more diverse metabolic capacities. Induced by bacteria, the [GAR+] prion state allows yeast to switch from purely fermenting glucose into a more versatile state that allows the simultaneous exploitation of diverse carbon sources (Jarosz et al., 2014a). Importantly, fitness of [GAR+] cells was found to be higher in low-glucose environments compared to cells in which the protein is in its non-prion state (Jarosz et al., 2014b).

In summary, epigenetic adaptation consists of a rich set of mechanisms that provide fascinating opportunities for organisms to rapidly disseminate variability in populations, long before genetic changes begin to fixate. But nonetheless, they are not heritable to the degree that genetic changes are. In the relay race context, there is a mutual effect between transcription activity and histone/DNA marks. As for the effect of epigenetics on later levels of the spectrum, it seems that the epigenetic architecture of genes also affects their chances of acquiring duplications or mutations (discussed below).

Adaptation by Changes in DNA Copy Number

As mentioned above, physiological and epigenetic adaptations are often carried out by changes in gene expression. Changes in DNA copy number are, in fact, another way to alter gene expression, yet this mode of adaptation is fundamentally distinct from the mechanisms mentioned above like transcription-factor-mediated changes. Genomic copy-number changes scale from single genes to aneuploidy (defined here as copy number change of whole chromosomes or parts of them). For most genes, a change in copy number results in altered mRNA levels as well as altered protein levels. This correlation between copy number and expression has been demonstrated in various organisms, including yeast (Dephoure et al., 2014; Pavelka et al., 2010a; Springer et al., 2010; Torres et al., 2007), plants (Huettel et al., 2008), mice (Kahlem et al., 2004; Lyle et al., 2004), and humans (Gao et al., 2007; Henrichsen et al., 2009; Stingele et al., 2012; Tsafrir et al., 2006; Williams et al., 2008). Therefore, DNA copy-number changes can be adaptive under selective pressures: when elevated expression is beneficial, extra copies can be acquired; conversely, when lower expression is beneficial, genomic copies can be lost. For example, the copy number of the human salivary amylase gene (*AMY1*) is positively correlated with the production level of salivary amylase protein, and populations with high-starch diets have more *AMY1* copies than those with traditionally low-starch diets (Perry et al., 2007).

When a higher expression of a specific gene is under selection, any genomic duplication that contains this gene has the potential to be adaptive. The most precise duplication would be of a small locus containing the gene in need. Yet, genomic adaptations are assumed to occur randomly, and thus the larger the duplicated region is, the higher the chances are for it to include the needed gene. For example, parallel *E. coli* populations evolved under limiting lactulose (a lactose isomer) showed duplication-based adaptations that varied in length. Although all duplication included the lactose permease (*lacY*), the shortest duplication covered 18 nearby genes and the largest consisted of up to 74 genes (Zhong et al., 2004). Notably, larger duplications come with a cost, as they contain many irrelevant genes whose expression is altered too. This altered expression of a large number of genes simultaneously imposes a significant burden on the cell (Bonney et al., 2015; Tang and Amon, 2013). Focusing on large copy-number variations like segmental aneuploidy or whole-chromosome aneuploidy (referred together as aneuploidy), it is important to note that despite their substantial cost, they have unique advantages and characteristics that distinguish them from the other forms of adaptation in the spectrum, as discussed below.

Aneuploidy as a Highly Accessible Evolutionary Solution

Aneuploidy is caused by mis-segregation of homologous chromosomes during cell division, and estimates indicate occurrence of 1:10,000 cell cycles in yeast and up to 1% in mammalian tissues (Knouse et al., 2014; Thompson and Compton, 2008; Zhu et al., 2014). Given these frequencies, populations of cells may constantly contain a variety of aneuploid cells that may be utilized as a resource for adaptation when facing a new challenge. Indeed, analysis of the yeast gene deletion library revealed that, in ~8% of the strains, deletion of a gene led to aneuploidy (Hughes et al., 2000). Interestingly, in some of the observed aneuploidies, the duplicated chromosome was found to harbor a close homolog of the deleted gene. In another study, a causal connection between aneuploidy and drug resistance was shown. The fungal pathogen *C. albicans* repeatedly acquired chromosome 5 aneuploidy in response to an antifungal drug exposure (Selmecki et al., 2008). The major mechanism by which duplication of chromosome 5 confers increased drug resistance is by amplifying two genes located in the duplicated chromosome: *ERG11* (encoding the drug target) and *TAC1* (encoding a transcriptional regulator of drug efflux pumps). Another yeast study showed that *S. cerevisiae* that have been evolved for ~200 generations under sulfate-limited conditions exhibited genomic duplications of regions that harbored the *SUL1* gene, which encodes a high-affinity sulfate transporter (Gresham et al., 2008). The rapid fixation of duplication-based adaptations mentioned above can be mainly attributed to their high occurrence in genomes and to the fact that duplications amplify many genes concurrently. This makes genomic duplications a highly accessible local maximum in the fitness landscape, whereas other adaptations are more complex and thus require longer evolutionary time to be acquired. An interesting hypothesis is that evolution acts to organize related genes on the same chromosome, perhaps even in proximity within the chromosome, so that duplications would elevate these genes together, with relatively fewer unrelated "hitchhiker" genes (Janga et al., 2008).

The Reversible Nature of Copy-Number-Based Adaptations

Aneuploidy-based adaptations are rapidly gained in evolution upon stress, but how reversible are such adaptations when the selection pressure is removed? The antifungal drug resistance that was facilitated by aneuploidy was shown to be reversible, as the extra chromosome was eliminated upon removal of the drug (Selmecki et al., 2006). In another study, yeast cells that were artificially selected for high expression of a single gene showed two types of distinct adaptations: duplication of large genomic regions (that contain the gene under selection) and *trans*-acting mutations. When selection was removed, only populations that adapted by aneuploidy could rapidly revert to base level (Rosin et al., 2012). This illustrates that adaptations based on duplications can serve as an "easy come easy go" adaptation, as when the stress is relieved, the costly duplication is driven out of the population much faster compared to sequence-based adaptations.

The Effectiveness of Aneuploidy for Acute and Abrupt Stresses

Genomic duplications appear to provide a rescue when a selective pressure is introduced in an abrupt manner, but would they appear also when stress is slowly aggravating? A recent lab evolution study (Yona et al., 2012) directly tested the effect of the stress regime, abrupt versus gradual, on the type of the selected evolutionary solution. This work demonstrated that, when yeast cells were abruptly shifted from 30°C to 39°C, where they evolved for some 500 generations, adaptation was repeatedly achieved by duplication of chromosome 3. Yet, populations that were evolved under a different heat regime in which temperature increased gradually (from 30°C to 39°C by +1°C increments every 50 generations) did not adapt by genomic

duplications but, rather, by sequence mutations. This suggests that, due to the high cost of aneuploidy, it is not an efficient response unless the selective pressure is acute and abrupt. Curiously, genome sequencing of the evolved populations shows that populations evolved under the abrupt heat-shock regime duplicated chromosome 3 but did not fixate any point mutation, while the populations that evolved under the gradual heat regime fixated 8–12 point mutations. It is tempting to speculate that this result could prove to be more general—that is, other conditions that select for aneuploidy under abrupt stress would select for changes other than aneuploidy when the same stress is applied gradually.

Genomic Duplications as a Transient Solution that Can Be Refined by Focal Adaptations

When the chromosome 3 aneuploid yeast were further evolved for additional >1,000 generations under high temperature, the extra copy of chromosome 3 was lost and replaced by a series of point mutations (Yona et al., 2012). The state of chromosome duplication thus appears as a transient step, an evolutionary "stepping stone." A similar evolutionary dynamic was observed in an *E. coli* study that showed how cells with impaired lac operon adapted first by multiple duplications of the impaired genes, as means to increase expression (Hendrickson et al., 2002). This amplification not only enabled lactose utilization, but also made the lac-operon hypermutable, as any additional copies increase the chances of finding beneficial mutations in this locus. Indeed, shortly after the gene amplification, one of the duplicated copies acquired mutations that restored a high functional level that led to the subsequent elimination of the other low-functional copies (Hendrickson et al., 2002). Interestingly, this dynamics may also be relevant to pathogens that adapt drug resistance in the clinics. A recent study on clinical isolates of *C. albicans* suggested that, in some isolates, aneuploidies may have an important role as an intermediate adaptation that subsequently gives rise to more stable adaptive genotypes that confer drug resistance (Ford et al., 2015). To conclude, it seems that prolonged evolution can solve the paradox of aneuploidy (Pavelka et al., 2010b; Sheltzer and Amon, 2011): Under normal conditions, selection purges fitness-lowering aneuploidy. Yet, under abrupt stresses, beneficial aneuploidy is selected because it confers higher survivability and proliferation to enable expansion of the effective population that can further search the fitness landscape for more optimal and slowly acquired solutions. Thus, aneuploidy appears as a transient step along the adaptation spectrum that facilitates a path to the next stage: sequence-based mutations.

Adaptation of the Genetic Sequence: Mutations on the Spectrum

The adaptation that is most commonly identified with evolution is genetic mutations. Mutations make the long-lasting adaptations, and unlike the previous stages along the adaptation spectrum, they can change not only expression regulatory regimes, but also actual protein sequence and function.

Adaptation through duplications or mutations could serve as alternative evolutionary strategies, but which genes go through which track? A recent study found that the answer could be in promoters' architecture. A careful analysis of nucleosome arrangement within promoters revealed a surprising deviation in some genes with the classical nucleosome-free region (NFR) architecture. It was found that genes whose expression is typically required at a certain level, like housekeeping genes, tend to have an NFR and low transcriptional plasticity i.e., low transcriptional variation across conditions (Tirosh and Barkai, 2008). It was further shown experimentally that the expression level of these NFR genes features low evolvability, i.e., their expression level is relatively insensitive to promoter mutations (Hornung et al., 2012). In contrast, genes that require a more dynamic transcription, like stress genes, typically lack an NFR, and their transcription is highly plastic and can be effectively altered by mutations (Hornung et al., 2012; Tirosh and Barkai, 2008). In a follow-up lab-evolution experiment (Rosin et al., 2012), yeast cells were put under short-term selection for higher expression of specific genes that were deliberately chosen to represent either cases of classical NFR or its absence. Interestingly, when the gene under selection had an NFR, selection toward higher expression was achieved by a large segmental or even whole-chromosomal duplications of regions that harbor the gene, presumably because of the low ability of these genes to elevate transcription by mutations. Conversely, for genes with no NFR, higher expression was achieved by mutations and with no duplications. This notion that nucleosome architecture of genes can create a bias that affects downstream adaptations on the spectrum (duplication versus mutations) highlights another relay race dynamic that connects between the epigenetic and genetic levels.

Here, we focus on genetic mutations only in the context of the adaptation spectrum and discuss some of the unique features of this adaptation mode. According to the "modern synthesis" of genetics and evolution, mutations are seeded at random in genomes, irrespective of environmental conditions or potential phenotypic effects. Nonetheless, there is some evidence suggesting that genetic mutations can be more accessible in challenging conditions and perhaps also in specific genomic regions.

Stress-Induced Mutagenesis and Transcription-Coupled Mutations

Diverse environmental stresses induce a higher rate of mutations (or lower efficiency of repair) (Gentile et al., 2011; Giraud et al., 2001; Loh et al., 2010; Oliver et al., 2000). In that respect, just like noise in gene expression or epigenetic noise in DNA methylation, mutations introduce diversity in populations especially under stress, when higher mutation rate may be beneficial. Subsequently, cells carrying mutations that confer higher fitness will prevail and improve the population's fitness. It is possible that stress-induced mutagenesis might not be adaptive, and it occurs simply due to the fact that, under stress, many processes in the cell are less accurate. Yet, indications on the precise control of error-prone DNA synthesis and some theoretical considerations on mutagenesis point toward the adaptive nature of increased mutagenesis under stress (Ishii et al., 1989; Lynch, 2010; Sniegowski et al., 2000). Mutagenesis, or DNA repair following mutagenesis, changes not only over time, but also spatially along the genome (Schuster-Böckler and Lehner, 2012; Supek and Lehner, 2015). Non-uniform distribution of mutations in different genomic regions suggests another potential

feature that may improve the efficient utilization of genomic mutations as a means of adaptation. What is truly interesting in our context is the process known as "transcription-coupled mutagenesis," by which the rate of mutation is elevated in proportion to transcription rate (Jinks-Robertson and Bhagwat, 2014). Thus, if transcription regulation allows "reading" different parts of the genome in different environments, transcription-coupled mutagenesis and related processes (Howan et al., 2012) may allow "(re)-writing," i.e., mutagenizing different parts of the genome at different rates under specific conditions. While previous chapters of this Perspective described protein and RNA inheritance of a Lamarckian nature, stress-induced mutagenesis and transcription-coupled mutagenesis both introduce some Lamarckian changes to the DNA level as well. Indeed, natural evolution seems now more Lamarckian than we thought until recently (Koonin and Wolf, 2009).

Phenotypic Mutations and Their Interaction with Genetic Mutations

Another means to diversify the proteome and the transcriptome before DNA mutations start to appear is known by the collective term "phenotypic mutations," representing errors in transcription and translation (Bürger et al., 2006). Of all processes in the Central Dogma, DNA replication often occurs with the highest fidelity. While error rate of DNA replication is typically between 10^{-8} and 10^{-10} per base per cell cycle, the error rate of transcription and translation, per nucleotide or per amino acid, could be up to a million times higher (Gordon et al., 2009; Meyerovich et al., 2010; Pan, 2013). Conceivably, phenotypic mutations should not propagate between generations, as the lifetimes of RNAs and proteins are typically short, even compared to the generation time of unicellular species. However, phenotypic mutations can propagate by triggering transcription network loop, by interaction with genetic mutations, or even by assimilation into the genome. A study on the lac operon of *E. coli*, which comprises an autocatalytic positive-feedback loop, has demonstrated a heritable epigenetic switch (Gordon et al., 2009, 2013). In this study, transcription infidelity generated a mutated lac repressor with reduced ability to repress the lac operon. This led the lac operon to be more sensitive to lactose, i.e., the operon could be induced by lower concentration of lactose. In such a case, a positive-feedback loop of the induced operon state of transcription is propagated across generations. Another interesting dynamic that involves phenotypic mutations lies within the interaction between phenotypic and genetic mutations. The "look-ahead mutations" concept (Whitehead et al., 2008) is a putative form of adaptation in which phenotypic mutations facilitate the fixation of high-complexity *genetic* mutations. Imagine, for example, that a new disulfide bridge between two cysteine residues is beneficial in a specific protein. Creating a new disulfide bond requires two genetic mutation events, in each of which a non-cysteine is converted into a cysteine. The evolutionary catch is that the organism's fitness does not increase before the *later* of the two mutation events occurs, i.e., since the first mutation alone would confer no (or even negative) fitness gain, it has lower chances of existing in the population. The "look-ahead mutation" is a theoretical scenario in which one of the two mutations is a phenotypic mutation, while the other is a genetic one. Under certain realistic quantitative assumptions (regarding error rates, etc.), it was shown that, indeed, a hybrid of phenotypic and genetic double mutant could emerge and be sustained. For example, a cell in the population that carries the first genetic mutation can obtain a second phenotypic mutation with partial functionality that will increase its fitness and, thus, its fraction in the population. Following that, the phenotypic mutation can be replaced with a fully functional genetic counterpart that will further increase the fitness (see Figure 3 in review by Koonin, 2012). In that respect, the phenotypic mutation may serve as an intermediate evolutionary "stepping stone" that can be rapidly attained and later replaced.

Phenotypic mutations might also be assimilated directly into the genome—for example, via the process of reverse transcription (RT). RT is not only used by retro viruses; it also occurs in cellular life, and it might act on genes in addition to retrotransposons (Cordaux and Batzer, 2009). This mechanism appears to be relevant in cancer, as a recent study found intron-less versions of human genes in cancerous genomes. These newly formed retro genes most likely result from reverse transcription of certain transcripts that are acquired somatically during cancer development (Cooke et al., 2014). Given the high rate of transcription errors, RT could serve as a potential evolvability mechanism by which phenotypic mutations become genetic.

Genetic Redundancy: The Longest-Lasting Adaptation?

We have proposed an adaptation spectrum that culminates in hard-wired genetic changes. Such changes are indeed stably "memorized" by genomes. However, even for a genetic adaptation, memory is not guaranteed to be indefinite. Mutational drift is certainly possible especially in periods when environmental conditions no longer necessitate the previously adaptive change. In this respect, what is more stable than a stable genetic change? Perhaps two stable genetic changes. Indeed, biological redundancy is prevalent in many genomes, and it is often suggested to provide a "fail-safe" mechanism, or backup: if one of two redundant genes is mutated, the other can still perform the lost function, albeit often upon a change in expression program (DeLuna et al., 2010; Kafri et al., 2009). The evolutionary stability of redundant genetic states is not trivial and can be sustained only under certain conditions (Nowak et al., 1997). Nonetheless, it is expected that, if the selective pressure that necessitated the adaptation is removed, the genetic adaptation will still be sustained, provided that the process of "neofunctionalization" (He and Zhang, 2005) has not yet taken place.

The processes that can occur along the adaptation spectrum have largely been described within the conceptual framework of one cell's lineage, and indeed we have been deploying the word adaptation in the context of an individual cell's (or organism's) improved fitness. However, to translate to evolutionarily meaningful changes and, indeed, to meet the more commonly recognized meaning of the term "adaptation," the described beneficial changes within a cell/organism need to ultimately result in changes at the population level. Our thinking on this is as follows. Consider an environmental stress that necessitates high expression of a particular gene. Cells in the population that highly express this gene, either in response to the stress or even prior to its occurrence, will have a temporary advantage over other cells. This higher expression may be achieved by stochastic differences at the physiological or epigenetic level. These

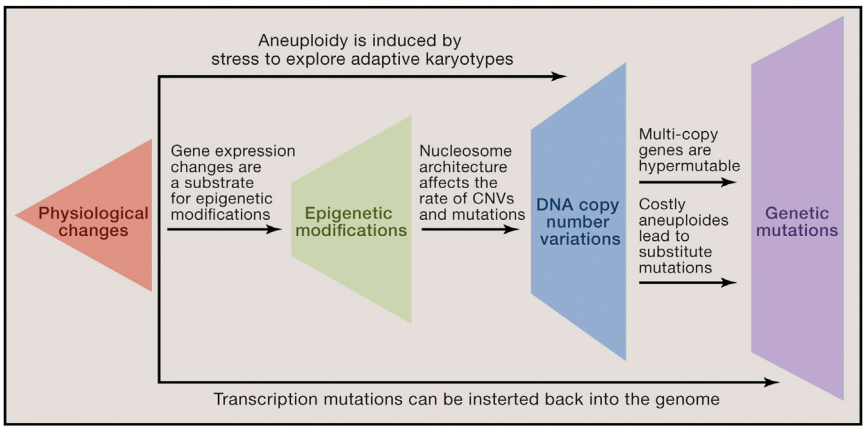


Figure 2. The Interplay between Different Modes of Adaptation on the Spectrum
Illustration of directional interactions between adaptation modes, where early adaptations with short persistence affect later, more durable, adaptations. Short descriptions of such interactions (arrows) demonstrate how later adaptations are more focused according to the trajectory set by earlier adaptations. We suggest that all modes of adaptation progress as in a relay race to optimize the whole process of organismal adaptation.

cells that exhibit higher expression have a dual advantage in the population: first, they benefit from a higher fitness (as long as the stress persists); second, due to relay-race dynamics, their lineages may have higher chances of propagating the original short-term acclimation into a more sustainable genetic adaptation. Such cell lineages will thus have an advantage both in terms of cell number and in terms of an increased per-cell probability of further adaptations. The outcome could be that, by the time the stress is relieved and the population returns back to its "ground state," the physiological acclimation has already been propagated to the genetic level and it now prevails in the population.

A Relay-Race Cascade within the Adaptation Spectrum

We have delineated an evolutionary adaptation spectrum along which organisms may progress as they adapt to a new challenge. We have taken the risk of generalization in suggesting a stereotypical order of exploration along the spectrum, starting from the physiological adaptation and gradually moving toward the genetic, though deviations from this simple-minded search strategy could certainly be envisaged. In this last section, we discuss the possibility that, in some cases, realization of a given stage along the spectrum could facilitate the progression into a next stage, as in a relay race (Figure 2).

Starting with the physiological level, changes in gene expression contribute to the first line of adaptation; nonetheless, they may also actively set in motion later modes of adaptation. It has been widely suggested that gene expression affects the epigenetic "chromatin landscape" (Henikoff and Shilatifard, 2011). For example, expression of a non-coding RNA induces epigenetic silencing of ribosomal genes by interaction with their promoter (Schmitz et al., 2010). Physiological changes in gene expression may also induce genomic duplications like aneuploidy. Interestingly, the higher rates of aneuploidy observed after stress are connected to the activity of the chaperone Hsp90 (Chen et al., 2012). In our context, Hsp90 may represent a relay-race baton that mediates between these two modes of adaptation. On top of being a stress-response gene, Hsp90 has an evolutionarily conserved role in the kinetochore assembly (Niikura et al., 2006), and therefore it also has a role in aneuploidy formation. Therefore, physiological modulation of Hsp90 under stress is suggested to facilitate a search for adaptations by diversifying the karyotype. Further down the spectrum, the classical role of Hsp90 can also be discussed in the context of the relay race. As a chaperone, Hsp90 was shown to act as an "evolutionary capacitor"—when active, it appears to mask the effect of mutations on the phenotype, and when repressed, those cryptic variations can be exposed (Rohner et al., 2013; Rutherford and Lindquist, 1998). In that respect, this protein serves as a baton in the relay race, mapping its own expression onto the effect of sequence mutation on the phenotype. Finally, physiological adaptations might also have an effect on genetic mutations. RNA transcripts (which typically carry more mutations) can serve as a template for DNA repair by homologous recombination of the original genomic sequence from which they were transcribed (Keskin et al., 2014) or, as mentioned above, by actual integration of a cDNA reverse-transcription product into the genome, as shown in cancer (Cooke et al., 2014). Such processes may facilitate rapid evolution of currently expressed genes, with a useful bias in favor of highly expressed genes, as they produce more RNA copies that can be reinserted into the genome.

Further down the spectrum, another relay-race dynamic occurs when genomic duplications promote subsequent genetic mutations. Large duplications (like aneuploidy) not only increase the mutation rate (Sheltzer et al., 2011), but also favor mutations that are related to the duplicated region. First, increased copy number increases the probability of a mutation in one of the duplicated copies. Second, the excessive cost of large duplications may affect mutations by favoring those who can replace the duplication. Any emerging mutation(s) that can replace the duplication-based adaptation is reinforced by an additional fitness increase at the magnitude of the cost that was saved. This added advantage increases the likelihood of such mutations to fixate faster than other mutations that are not related to the initial duplication. In this way, there is a bias on subsequent mutations to cope with the selective pressure that led to the initial duplication-based adaptation.

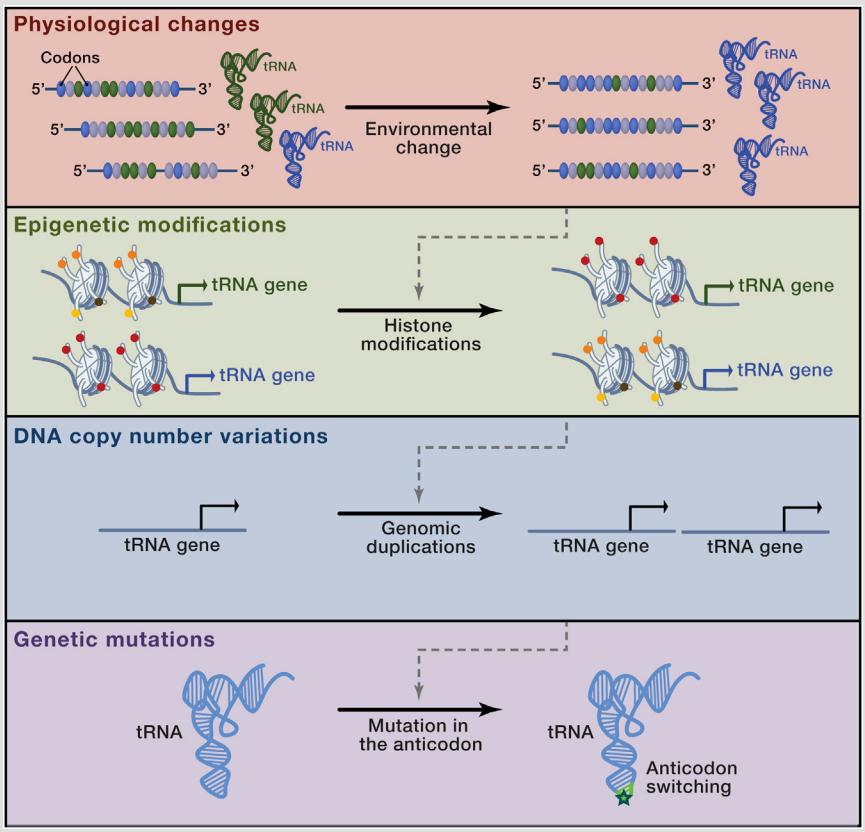
We present the example of translational optimization with which progression along the adaptation spectrum can be conceptualized with a specific set of cellular processes in Box 1.

A major topic that has not been discussed here in great length is that of cancer. Cancer is an evolutionary process, and as such, it may exploit different adaptions along the spectrum, as well as the relay race between them. To begin with, the cancerous transcriptome is known to be radically reprogrammed (c.f., Segal

Box 1. The Adaptation Spectrum of the Translation Process: A Test Case

Many of the cellular resources, i.e., energy and raw materials, are devoted to ensure adequate translation of proteins. Consequently, translation optimization is a major driving force in evolution. One of the factors that governs translation optimization is the balance between supply and demand i.e., between the tRNA pool to the codons used by currently expressed mRNAs. Perturbations in the supply-to-demand balance emerge when a new environment requires the expression of different proteins with a different codon usage (Gingold et al., 2012) or because the availability of some tRNAs is altered (Dittmar et al., 2005; Pavon-Eternod et al., 2013; Wiltrout et al., 2012). Examining how cells restore translational balance reveals many of the stages along the adaptation spectrum.

Measuring expression of the tRNA pool under diverse conditions shows physiological response in which distinct tRNA types are up/downregulated (Gingold et al., 2014). On the next level, epigenetics is becoming increasingly appreciated in this context, as histone modifications around tRNA genes change dynamically in response to cells' conditions (Barski et al., 2010; Gingold et al., 2014; Oler et al., 2010), e.g., in cancer. Further on the spectrum, duplication of tRNA genes appears to provide elevated expression from certain tRNAs that are in high demand, and indeed, many of the tRNA genes occur in multiple-copy families that change their relative sizes in evolution (Man and Pilpel, 2007). Therefore, it will be interesting to ask whether some of the chromosome gains and losses observed in cancer correspondingly increase or decrease tRNAs' availability to support the progression on cancer. Next, mutations within tRNAs were also found to be adaptive, presumably in response to change in the demand-to-supply ratio. When a tRNA gene is artificially deleted from the yeast genome, another tRNA gene with a different anticodon but of the same amino acid evolutionarily "responds" with a mutation that converts its anticodon to that of the deleted one (Yona et al., 2013). Such "anticodon switching" was subsequently found to be very prevalent in the natural evolution of species (Rogers and Griffiths-Jones, 2014; Yona et al., 2013). In each of these cases, we do not know whether the anticodon-switching mutation was preceded by earlier transcriptional/epigenetic changes, yet it is tempting to speculate that such physiological changes may have constituted an intermediate solution to the challenge before it was solved genetically. Further, the tRNA pool probably also realizes the last stage of the adaptation spectrum, i.e., that of genetic redundancy by compensation over mutated tRNAs (Bloom-Ackermann et al., 2014). Thus, partial redundancy among tRNAs may act to increase evolutionary stability on one hand and to facilitate evolutionary plasticity of the tRNA pool on the other hand.



The green and blue ovals represent codons that correspond to the anticodons of the green and blue tRNAs. Prior to an environmental change (upper-left), there is a high usage (translation demand) of a certain codon (green oval) compared to another codon (blue oval), and the tRNA levels (translational supply) match accordingly. An environmental shift (upper-right) may result in a physiological change both at the codon usage (now, the blue codon is in higher demand, because mRNAs that are enriched in the codon are induced), and tRNA levels adjust correspondingly. The higher expression of the blue tRNA could then be propagated into the epigenetic level, e.g., through changes in activation or repression-associated histone mark in the tRNA genes' vicinity. Such changes in the tRNA pool may be further implemented by changes to tRNA gene copy number. Finally, more copies of the same tRNA gene may increase its probability of acquiring both functional and regulatory mutations, like anticodon switching. Dashed arrows (gray) connecting the different levels represent hypothesized relay race between the levels.

et al., 2004), and recent analyses of cancer epigenomes showed that DNA methylation is stochastic rather than precise (Landan et al., 2012; Landau et al., 2014). The question of whether these changes at the physiological and epigenetic levels are connected remains unknown. Further along the spectrum, aneuploidy is a hallmark of cancer, and despite the debate of whether it is a cause or a consequence of cancer (Sheltzer and Amon, 2011), aneuploidy is suggested to provide cancer with both higher mutation rate and with a faster means to change dosages of cancer-driving genes, i.e., upregulating expression of oncogenes or downregulating tumor-suppressor genes (reviewed by Gordon et al., 2012). Furthermore, since aneuploidy can

increase copy number of cancer-driving genes and mutation rates simultaneously, it may lead to a hypermutability effect of oncogenes that were duplicated. Taken together, it is intriguing to speculate that cancer cells might exploit the relay race notion proposed here in gaining more aggressive traits much faster.

In conclusion, we suggest that the distinct modes of adaptation have been optimized by evolution not only to perform their adaptive function, but also to interact with later modes of adaptation. In this way, the whole process of adaption can yield better results as the fitness landscape is being explored more efficiently according to the trajectory set by earlier adaptations.

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