Leading Edge
Review

Cell

Timescales of Genetic and Epigenetic Inheritance

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According to classical evolutionary theory, phenotypic variation originates from random mutations that are independent of selective pressure. However, recent findings suggest that organisms have evolved mechanisms to influence the timing or genomic location of heritable variability. Hypervariable contingency loci and epigenetic switches increase the variability of specific phenotypes; error-prone DNA replicases produce bursts of variability in times of stress. Interestingly, these mechanisms seem to tune the variability of a given phenotype to match the variability of the acting selective pressure. Although these observations do not undermine Darwin's theory, they suggest that selection and variability are less independent than once thought.

Introduction

In 1943, by plating a number of independent bacterial cultures onto lawns of infectious phages, Salvador Luria and Max Delbrück showed that each bacterial population contained a widely variable number of phage-resistant mutants (Luria and Delbrück, 1943). Hence, they argued, these mutants must have been generated prior to the phage infection and not in response to the infection, as that would likely produce a comparable number of mutants in each culture. The apparent independence of variation and selection confirmed a cornerstone of the classic Neo-Darwinist theory of evolution. In contrast to Darwin's original theory, the Neo-Darwinist theory firmly rejects Lamarck's idea that organisms pass on characteristics they develop during their lives (Weismann, 1893). The Neo-Darwinian idea that evolution is driven by purely random germline mutations followed by independent natural selection on the progeny has become a widely accepted dogma in biology.

The resulting focus on mutation as the mechanism for phenotypic variation has led to detailed measurements of mutation rates. In addition, genotype-to-phenotype mapping became one of the major focuses of the molecular biology revolution. Many studies have defined the stability, which is generally measured as the rate of change of the phenotype per cellular generation, of various phenotypes. Notably, this massive research effort has identified phenotypes whose stability differs significantly from typical phenotypic stabilities (Figure 1). For example, certain phenotypes are inherently less sensitive to mutation, and this insensitivity of a phenotype to genetic mutation is often referred to as "robustness" or "canalization" (Waddington, 1942). By contrast, other phenotypes exhibit unusually rapid variation due to underlying hypervariable sequences in the genome (Srikhanta et al., 2005; van der Woude and Baumler, 2004). Still other phenotypes exhibit rapid variation despite no underlying genotypic change; these phenotypes belong to the class of "epigenetically" heritable phenotypes (for a review, see Jablonka and Lamb, 1995). These and many other examples demonstrate that phenotypic stability spans many orders of magnitude beyond the range expected from classic genetic mutation studies, with some phenotypes varying rapidly while others are unusually stable (Figure 1).

Like phenotypic changes, changes in the selective pressure acting upon organisms also occur over an exceptionally broad timescale. Some changes, such as temperature changes and periods of famine, may occur within an organism's life span (one generation). Geological changes, on the other hand, span several thousands or even millions of biological generations. The ability of organisms to change phenotypes to cope with changing environments during their lifetime is known as "plasticity." For geological timescales, phenotypic change mostly occurs by sequence evolution, and the ability to effect this change is called "evolvability." However, environments (and thus selection) change over timescales intermediate to these two. For example, predator-prey cycles, cyclical climate changes such as El Niño, and battles between infectious microbes and their host's immune system may all act on timescales greater than one generation but shorter than geological timescales of thousands of generations.

This Review addresses the timescales, over which heritable biological phenotypes vary, and gathers examples

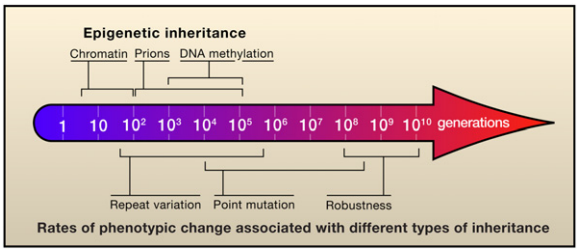

Figure 1. The Timescales of Inheritance

Both inheritance and selection can act on a wide array of different timescales, ranging from fewer than one cellular (or organismal) generation to more than one billion generations. A number of different mechanisms exist that regulate the stability of biological phenotypes. Phenotypes inherited epigenetically often exhibit rapid variation, whereas genetically robust phenotypes are stabilized against random mutation. Here, we show rough timescales, in units of cellular generation, for the stability of phenotypes regulated by the indicated mechanisms.

of biological mechanisms that are seemingly designed to regulate or at least influence the timing or location of phenotypic variation. Where possible, we will explore the correlation between the variability of a given phenotype and the variability of the selective pressure that is proposed to act upon it. More specifically, we will argue that organisms appear to have developed mechanisms to tune the timescale of their own heritable variability to match the timescale of the acting selective pressure. For example, pathogenic organisms often exhibit rapid variation in the expression of cell-surface molecules that might be recognized by the immune system and which switch between different expression states as rapidly as every 50 generations. In this case, rapid switching is likely to provide the pathogen with a way to escape immune responses, with the antigenic switching rates tuned to the timescale of the host immune response (for a review, see van der Woude and Baumler, 2004). Such mechanisms contradict the total randomness of heritable variability, which is one of the foundations of today's generally accepted theory of evolution.

This subject can be construed extremely broadly, and we note some intentional limitations to our Review. First, we will focus our Review on unicellular organisms, as their rapid generation time and high population sizes have enabled the experimental study of rare phenotypic changes. We will, however, discuss selected examples of related phenomena of interest in multicellular organisms. It is also important to note that for many of the phenotypes discussed, detailed studies of selective pressure in ecologically relevant environments are sparse, so any discussion regarding temporal variation in selective pressure is largely speculative by necessity.

Before we elaborate on some of the examples where the timing or location of variability is regulated by complex genetic or epigenetic mechanisms, it is useful to first consider random sequence mutation, which is arguably the most common mechanism for phenotypic change.

Mutation Rates and Target Size

The best understood mechanism for phenotypic change operates via change in DNA sequence. Point mutation rates vary between organisms, and values range up to about 10^{-4} per base pair per generation for certain RNA viruses, around 10^{-6} to 10^{-8} for most microbes, and 10^{-9} per base pair per cellular generation for human cells. In general, mutation frequencies increase with increasing population sizes and decreasing information content of the genome, which results in a surprisingly stable mutation rate of roughly 1/300 non-neutral mutations per genome per generation (Drake, 1999). However, matters are complicated by the fact that mutation rates vary across the genome. Early studies on the *Escherichia coli* Lac repressor, for example, revealed significant mutation-rate variation across the gene (Miller et al., 1977), while recent genomic studies on silent site mutations in humans revealed hot spots and cold spots that cover hundreds of kilobases (Chuang and Li, 2004). The reason for variation in mutation frequencies in the complex human genome is poorly understood. In the much simpler genomes of bacteria, some mutational hot spots have been linked to special DNA sequences such as inverted or tandem repeats (see below).

Even if mutation rates were uniform across the genome, not every phenotype would vary at the same rate because of differences in the so-called "target size" of the phenotypes. As an illustrative example, consider a phenotype that depends on the function of several proteins, including a massive protein with many essential amino acids and a required C-terminal domain. This phenotype will be lost if any of the essential amino acids are mutated in any of the proteins or if mutation to a premature stop codon prevents the required C terminus from being expressed. Conversely, a phentoype that depends solely on one small protein with few vital domains presents a much smaller target size. Target size cannot be calculated from sequence; it obviously depends very strongly on which proteins are required for the phenotype in question, which amino acids are essential for the proteins' function, which codons are used by these amino acids, and many other factors. Hence, it is difficult to estimate the precise impact of the target size on phenotypic variability. Perhaps advances in computational protein-structure prediction will enable some intuition concerning target size for the misfolding of arbitrary proteins, and functional genomic studies may identify the number of proteins required in a given pathway.

While mutation rate and target size are somewhat difficult to measure, the product of the two can be directly measured and is given (for traits that can be scored as present or absent) as the rate of gain/loss of a phenotype per generation due to mutation. For example, haploid yeast mutants lacking orotidine 5′-phosphate decarboxylase (uracil biosynthesis) occur at $\sim 10^{-7}$ per generation (Boeke et al., 1984). For continuously varying "quantitative traits," the experimental correlate of mutation rate times target size is the mutational variance Vm of a phenotype.

Vm is defined as the per-generation increase in the mathematical variance of a quantitative trait across a population due to random, unselected mutations. Mutational variance is typically measured by allowing a broad spectrum of unselected mutations to accumulate by passaging individuals of a species independently at very small population sizes (eliminating any but the strongest effects of selection), followed by measurement of the phenotype of interest.

Given a mutation rate and a target size, one may, in principle, predict the stability of a phenotype of interest. However, researchers have discovered several cellular mechanisms that increase or decrease the rates of change of a subset of phenotypes. It is useful here to distinguish between regulation of global variation, locus-specific variation, and temporal regulation of variation (local or global; Jablonka and Lamb, 2005; Metzgar and Wills, 2000). The broad idea that cells have evolved the ability to regulate the global tempo of phenotypic change is irrefutable. The existence of proofreading activities and sophisticated error-correction systems encoded in most genomes demonstrates that evolution has selected for systems that modulate the fidelity of information transfer between generations. Indeed, subpopulations of cells lacking proofreading activities, known as "mutators," are found at high frequencies (often on the order of 1%) in microbes gathered from the environment (LeClerc et al., 1996). However, we aim specifically to discuss examples of localized variation in the fidelity of information transfer (genotypic or, in some cases, exclusively phenotypic). We will also discuss mechanisms that regulate the timing of variability, with cellular stress generally leading to increased variation. Finally, we describe a few examples where cells are able to influence both the timing and location of variability in response to environmental cues.

Localized Variation

Contingency Loci and Rapid Genotypic Variation

Analysis of mutation rates in the *E. coli* Lac operon showed that many mutation hot spots corresponded not to base substitutions but to insertions and deletions in short repeated sequences (Farabaugh et al., 1978). Since then, numerous examples have been described of rapid sequence change associated with hypervariable DNA loci, termed "contingency loci" (for a review, see van der Woude and Baumler, 2004). Through various mechanisms, these loci are unusually prone to specific types of mutations that result in the alternating on- and off-switching of specific genes. Switching between the two resulting phenotypes (called "phase variation") enables organisms to quickly adapt to frequent and recurring changes in the environment. Switching frequencies as high as 10^{-1} have been reported, although frequencies on the order of one switch in every 10^3–10^5 generations are more common (van der Woude and Baumler, 2004).

The best known examples of contingency loci are in bacteria. The term "contingency locus" was first coined to describe the reversible promoter that controls the *Salmonella* flagellar synthesis genes (Simon et al., 1980). The promoter is surrounded by inverted repeats, which are subject to frequent recombination events that result in promoter inversion. When the promoter inverts, the expression of one flagellar gene is arrested, and a second gene on the other side of the promoter is activated. Although the precise biological function of this phase variation remains to be demonstrated, the expression of two different flagellar antigens may help to evade the host immune system and/or to infect different tissues (van der Woude and Baumler, 2004). Many other contingency loci have been described, mostly in pathogenic microorganisms, where hypervariable loci commonly control the expression of cell-surface antigens. A special case is that of the trypanosomes, which contain an arsenal of about 1000 silent "variant surface glycoproteins" (VSGs). Only the one gene localized in the active VSG expression site is transcribed. By regularly replacing the VSG gene in the active expression site, the parasites constantly switch their outer surface coat (Barry and McCulloch, 2001).

Another interesting case of contingency loci is found in the common brewer's yeast *Saccharomyces cerevisiae*. Many *S. cerevisiae* cell-surface genes contain tandemly repeated DNA sequences in their coding sequences (Verstrepen et al., 2004, 2005). The repeats are subject to frequent recombination events, which often result in repeats being gained or lost (Figure 2). One such gene, *FLO1*, encodes a cell-surface protein that enables yeast cells to adhere to various substrates. Cells carrying a greater number of repeats in *FLO1* show a stronger adherence to plastic surfaces such as those used in medical devices. Repeat variation may therefore allow fungi to rapidly attune their cell surfaces to new environments. It is interesting to note that in this case, the repeats do not cause switching of expression states in a repertoire of cell-surface genes. Instead, unstable intragenic repeats generate limited changes in a small set of expressed proteins. Similar repeat variation in genes of pathogenic fungi may contribute to the cell-surface variability needed to evade the host immune system (Verstrepen et al., 2005).

Although they are usually not referred to as contingency loci, similar hypervariable loci are also found in metazoans, including humans (where they are often associated with diseases). Classic examples include neurodegenerative diseases, such as Huntington's chorea and fragile X syndrome, where expansion of intragenic repeats leads to malfunction of the associated gene. The timescale of these expansion/contraction events has been extensively studied in fragile X syndrome, where the rate of repeat expansion varies depending on the sex of the carrier and the initial (pre-existing) number of repeats: in females carrying alleles with 90–100 repeats, up to 87% of the offspring inherit a disease-causing full mutation (>200 repeats). This rate drops to ~5% for the offspring of mothers carrying between 55 and 59 repeats, whereas mothers with fewer than 55 repeats never pass on the full mutation to their children (Nolin et al., 2003). Interestingly, at many of these repeat-containing genes, repetition is highly conserved

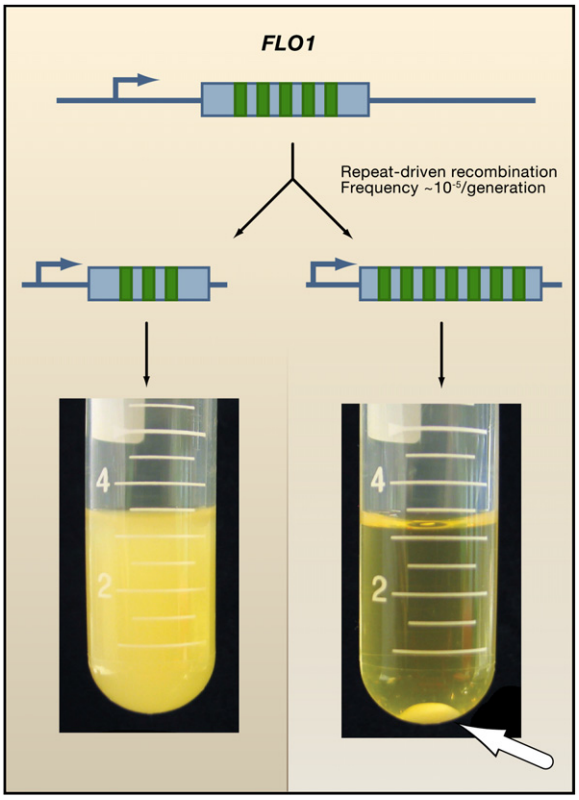

Figure 2. Recombination in Intragenic Repeats

Certain genes, such as the *S. cerevisiae FLO1* gene, contain tandem repeats within their coding sequences. These repeats are highly unstable and recombine at frequencies around 10^{-5} per (mitotic or meiotic) generation, resulting in the net loss or gain of repeat units. If the repeat units are not a multiple of three nucleotides, recombination gives rise to frameshifts, resulting in switching on and off of the gene. Most repeats found within open reading frames, however, are a multiple of three nucleotides long. In this case, recombination results in longer or shorter alleles of the protein. The length variation can have functional consequences. In *FLO1*, for example, longer alleles confer flocculation (i.e., the adhesion of yeast cells to each other to form a "floc" of cells that sediments in the medium; white arrow). Short *FLO1* alleles confer gradually weaker flocculation, with the very shortest alleles resulting in completely planctonic (suspended) growth.

not only of amino acid sequence but also at the DNA level, which suggests the possibility of a beneficial outcome to some rapid repeat variation that offsets the disadvantages caused by pathogenic repeat variation (Verstrepen et al., 2005).

An interesting example of repeat variation that could conceivably prove beneficial in a population is found in a tandem repeat region upstream of the vasopressin receptor gene *Avpr1a*, which is known to influence sociobehavioral traits in voles (Hammock and Young, 2005). The repeat locus is highly variable in populations, which suggests an elevated mutation rate compared to that of other genomic regions (though the per-generation rate of repeat variation was not directly measured). Phenotypically, expansion of this repeat region increases promoter activity, and males with more repeat copies in the *Avpr1a* promoter show increased caretaking for their pups and increased pair bonding with partner females compared to individuals with fewer repeats. This repeat variation could therefore allow for rapid evolution of behavioral traits that may be of adaptive benefit in different environments. A second example of repeat-associated phenotypic plasticity that is seemingly not pathogenic was found by Fondon and Garner (Fondon and Garner, 2004). These authors demonstrate that repeat variability in the coding regions of the *Alx-4* (aristaless-like 4) and *Runx-2* (runt-related transcription factor) genes is associated with quantitative differences in limb and skull morphology in dogs. Hence, these repeats may allow rapid evolution of morphological variants on a conserved basic body plan that may provide an adaptive advantage as the selective environment changes.

Epigenetic Inheritance and Rapid Phenotype Switching

Another class of phenotypes vary at rates similar to, or often even higher than those typically generated by contingency loci. In most cases, this variation does not rely on mutations in the DNA sequence but rather relies on alternative, so-called "epigenetic" methods of inheritance. Like contingency loci, epigenetically heritable traits typically exhibit a limited repertoire of phenotypes and interconvert ("switch") more rapidly than do phenotypes that change by point mutation. Epigenetic switches can be grouped according to the mechanism of inheritance, as epigenetic information is carried by substrates ranging from DNA methylation patterns to the folding of prion proteins.

Methylation of DNA bases is one of the major mechanisms of epigenetic inheritance and has been implicated in phenotypic inheritance in unicellular organisms, in cell-state inheritance in multicellular organisms (during one organismal generation), and in transgenerational inheritance in multicellular organisms. For example, although some phase variation in bacteria is due to changes in genomic sequence (above), other cases rely on epigenetic inheritance of methylation patterns. One of the best studied examples is found in control of the pyelonephritis-associated pili (pap) operon by DNA methylation (Hernday et al., 2002). Here, the on and off states are distinguished by methylation of Lrp-binding sites found proximal and distal, respectively, to the *papBA* promoter. The switch from on to off occurs at $\sim 10^{-4}$ per generation, whereas the converse switch occurs at $\sim 10^{-2}$ per generation. An interesting example of heritable methylation-mediated phenotypic variation in multicellular organisms is in the flowering plant *Linaria vulgaris*. Naturally occurring variation in methylation of the *Lcyc* gene distinguishes "peloric" morphological mutants with radial floral symmetry from the wild-type variant with bilateral floral symmetry (Cubas et al., 1999). The accelerated phenotypic variation due to this "epimutation" may be adaptive in the context of the rapid timescale of plant-pollinator coevolution.

Another classic example of epigenetic inheritance is the silencing of subtelomeric genes in microorganisms. Yeast

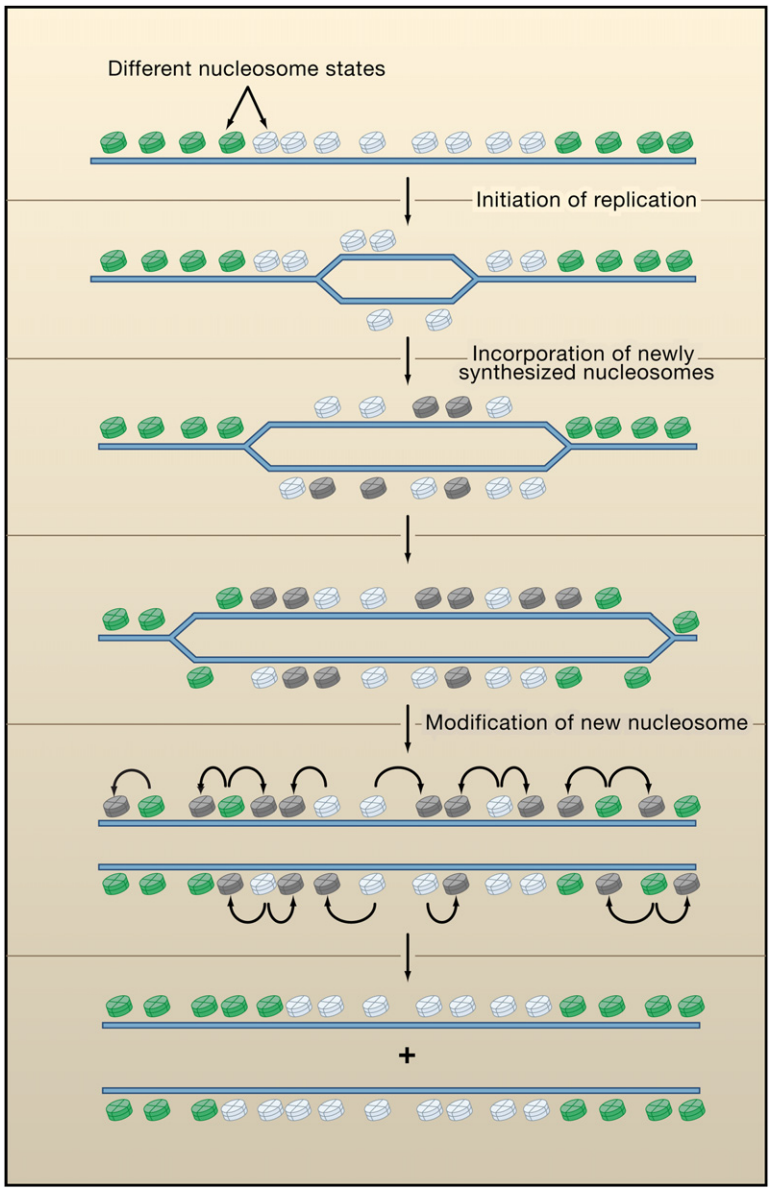

Figure 3. A Model of the Inheritance of Chromatin States

Chromatin states have been proposed to carry heritable epigenetic information. Shown in green and white are two different nucleosome states (possibly carrying distinct covalent modification patterns or distinct subsets of variant histone isoforms). After passage of the replication fork, nucleosomes apparently segregate randomly to the two daughter chromosomes. Soon thereafter, newly synthesized nucleosomes (gray) are assembled onto the chromosomes. In order for chromatin states to be heritable for more than a handful of generations, new nucleosomes must be modified to the same state as surrounding maternal nucleosomes. In one possible model of this feedback the proteins that associate with maternal nucleosomes locally instruct (arrows) new nucleosomes to carry the appropriate modification/variant pattern. This model is one of several proposed, but all models have in common some feedback mechanism by which old nucleosomes influence the states of the newly synthesized nucleosomes deposited at a given locus.

telomeric regions contain multiple gene families, including the cell-surface *FLO* genes, the thiamine-biosynthesis *THI* genes, and the hexose kinase *HXK* genes. Genes located near telomeres are subject to variegated silencing; for example, a reporter gene adjacent to an artificially constructed telomere was shown to switch from on to off approximately every 10 to 15 generations (Gottschling et al., 1990). Two related histone deacetylation mechanisms are responsible for subtelomeric silencing: genes immediately proximal to the telomeres are silenced by the silent information-regulator (Sir) complex, whereas genes located somewhat more distant are silenced by Hda1 (Gottschling et al., 1990; Halme et al., 2004). Although the linkage between histone deacetylation and silencing is well established, the mechanism of inheritance of chromatin states (both on and off) is still an active area of investigation (Figure 3). A similar phenomenon occurs in the malaria pathogen *Plasmodium falciparum*, where virulence factors such as the erythrocyte-adhesion molecule PfEMP1 are encoded subtelomerically and vary in expression from on to off approximately every 50 generations (Roberts et al., 1992) in a Sir2-dependent manner. Stochastic subtelomeric switching of cell-surface genes of pathogens may help evade the host immune system, and presumably switching rates are tuned so that the time of exposure of an antigen is shorter than the time required for an effective immune response.

Prions (proteins that can heritably occur in more than one conformation) are fascinating examples of epigenetic information carriers that are stable for relatively long timescales. Prion proteins were originally described as infectious protein conformations that convert the

normal host protein into the prion form. However, in some cases, prion forms appear to be transmitted from mother to daughter cells, and the evolutionary conservation of prion-forming domains suggests that this ability can be beneficial. In yeast, translational readthrough of stop codons is caused by the aggregated prion state (called [PSI^+]) of the translation termination factor Sup35 (Uptain and Lindquist, 2002). Sup35 can aggregate in a variety of prion conformations, thus leading to a range of phenotypes characterized from "weak" to "strong" prion states and complicating the characterization of switching rates. However, it is clear that prion states are more stable epigenetic states than subtelomeric expression states. The *S. cerevisiae* [PSI^+] state, for example, is stable for approximately 10^5 to 10^7 generations (Lund and Cox, 1981).

In multicellular organisms, a great deal of recent effort has focused on the role of transgenerational inheritance of RNA molecules. Most notably, microinjection of double-stranded RNAs into *Caenorhabditis elegans* is sufficient to produce a loss-of-function phenotype in a substantial fraction of F2 animals, and this effect persists for up to 80 generations after the injection (Fire et al., 1998; Vastenhouw et al., 2006). A small number of molecules were sufficient to initiate this heritable effect with thanks to amplification of the interfering RNAs by RNA-dependent RNA polymerase. In mammals, epigenetic inheritance of RNA molecules was recently described in which expression of unusual *Kit* RNAs in the germline of mice resulted in a phenotypic effect (on coat color) in the progeny of the affected mice such that two genetically identical mice might differ phenotypically based on their parents' genotypes (Rassoulzadegan et al., 2006). This last example mirrors the phenomenon of paramutation in plants, which was first discovered in maize in the 1950s by R. Brink (see also the Essay by V. Chandler, page 641 of this issue).

Other mechanisms of epigenetic inheritance are even more cryptic than the examples above. In bacteria, persistence to antibiotic treatment is characterized by a small subpopulation that grows slowly and is not killed by antibiotic treatment (Balaban et al., 2004). These slowly growing bacterial "persister cells" are a rare phenotypic subpopulation, and the majority of progeny of these persisters revert to the sensitive but rapidly growing phenotype (Balaban et al., 2004). Actively growing *E. coli* switch to slowly growing persisters at a frequency of approximately 10^{-6} (per hour, not generation), whereas persisters generate actively growing progeny at a frequency of 10^{-1} per hour. The mechanism for this phenotypic switch is unknown, although mutants with changed switching frequencies have been identified, and the genes affected may provide clues as to the substrate for this phenotypic switch.

We expect that more examples of epigenetic switches are likely to be found. Generally, switching between two semistable states ("bistability") is a common property of (genetic) networks with positive feedback loops (Rao et al., 2002). By definition, bistability allows two stable states to exist in the same environment. In addition, bistable systems often exhibit hysteresis, which means that the most likely state of the system in a given environment is influenced by the past history of the organism. For example, classic studies on the *E. coli* Lac operon identified conditions under which the transcriptional response to intermediate levels of lactose was both bistable and hysteretic (Novick and Weiner, 1957). In other words, transcription of the operon occurs at one of two levels, and, at intermediate lactose concentrations, the level of expression is determined by the past history of the cell. This cellular memory is stable for several generations after cells are shifted to the intermediate inducer level, thus providing an example of an epigenetically heritable network state. Similar short-term inheritance of a memory state in intermediate inducer concentrations is also present in other regulatory systems, such as an experimentally modified yeast *GAL* network (Acar et al., 2005), and is predicted to be a common feature of complex cellular networks with feedback loops.

Why Switch Stochastically?

As is clear in the examples above, traits associated with contingency loci and epigenetic switching typically alternate between a limited number of phenotypes or "states," such as the on- and off-expression states of subtelomeric genes, the radial and bilateral floral symmetries, or the various repeat numbers in cell-surface proteins. This raises the question of why organisms have developed such complex switching mechanisms to reach the seemingly simple goal of turning genes on and off. Is it not more straightforward to use plasticity, e.g., transcriptional regulation, to switch between a handful of phenotypic states? The adaptive benefits conferred by stochastically fluctuating phenotypes have been the subject of a number of modeling studies (Jablonka et al., 1995; Kussell and Leibler, 2005; Wolf et al., 2005). In general, these studies suggest that random, heritable phenotypic switches may be beneficial when the environment fluctuates randomly over timescales that are roughly matched to the phenotypic switching rate. Several of these studies also explicitly compare stochastic switching with plasticity by modeling (1) the costs associated with maintaining sensing machinery and (2) the time delay between sensing and phenotypic change. For example, an environment that proves instantly lethal cannot be dealt with by plasticity. Together, these results demonstrate that some environments and sensor regimes exist for which stochastic switching is the optimal organismal "bet-hedging" strategy. Environmental regimes where conditions persist for at least several generations, but not tens of thousands of generations, are expected to select for stochastic phenotypic variation on timescales not typically accessible to point-mutational processes. Interestingly, for many of the examples described above, it can indeed be argued that the switching frequencies could match the variability of the selective pressure that is acting upon the respective phenotype (see further).

Of course, random switching comes at a cost: it results in some maladapted individuals in every generation. A

directed switching strategy in which cells bias their progeny phenotypes based on the recent environment would be preferable (Jablonka et al., 1995). This inheritance strategy, which is widely disbelieved (but experiencing a recent resurgence), is now often referred to as "Lamarckism" and will be addressed at the end of this Review. We first turn to the decrease of variability in certain phenotypes.

Robustness and Canalization

The phenomena described above are all examples of mechanisms that increase the rate of phenotypic change beyond the rate due to random mutation. Conversely, many phenotypes have proven beneficial to cells over countless generations through many environments. Organisms might therefore have evolved mechanisms to stabilize these traits against the random degradation of undirected mutation. Phenotypes stabilized in the face of genetic mutation are known as *genetically* robust and should be separated from traits that are stable in a wide range of environmental regimes, which are *environmentally* robust. The idea that a phenotype could be the result of many genotypes (and hence stable to mutations that change one genotype to another) has been described as "canalization" (Waddington, 1942), "buffering," or "robustness." For quantitative traits, robustness can be defined using the mutational variance Vm (see above): if Vm for phenotype P is lower in organism A than in organism B, then organism A is more genetically robust than B.

There are a number of ways that individual genes may be robustly encoded. For example, several amino acids are encoded by multiple codons, and these codons may differ in the number of mutations that change the encoded amino acid. For example, CGA, CGC, CGG, CGT, AGA, and AGG all code for arginine. Mutation of the third base for any of the CG**X** codons will not change the amino acid encoded, whereas mutation of the third base of AGA or AGG may change the protein sequence. Encoding arginine with CG**X** thus reduces the mutational target size of the protein (assuming that arginine is essential for the protein's function) by about one nucleotide. One study discussed this property as "codon volatility" and suggested that genes under stabilizing selection are generally encoded by low-volatility codons (Plotkin et al., 2004). However, it is currently unclear whether this enrichment reflects some correlated property of codon bias that is selected for some reason besides robustness. In any case, it is intuitive that decreasing a phenotype's mutational target size will stabilize a phenotype against mutation. Perhaps a more obvious and widespread mechanism to establish robustness of certain traits is gene duplication, where the second gene copy can provide a "backup" system when one copy is mutated.

Another example of robust encoding has been described for RNA secondary structures (Ancel and Fontana, 2000). A number of different RNA sequences are capable of folding into a given secondary structure. Some RNA sequences are more genetically robust than others in the sense that fewer mutations will prevent appropriate folding. Interestingly, using in silico folding predictions, the authors found that RNA sequences that are capable of folding into a given structure at a wide range of temperatures are also less prone to change their structure as a consequence of mutations. This case therefore provides an example of a mechanism for the evolution of robustness known as "congruent robustness," where genetic robustness may occur as a side effect of selection on environmental robustness (Ancel and Fontana, 2000).

At a more global level, it has been suggested that organisms have evolved mechanisms to increase the genetic robustness of complex phenotypes (such as body plan) to protect vital phenotypes from genetic insults. This was first discussed in the seminal work of Waddington (Waddington, 1942, 1953), who noted the exceptional stability of organismal development in the face of environmental perturbations and genetic mutations. He suggested that deep "canals" seemingly direct the developmental flow and called the process canalization. This idea has its echoes today in the systems-biology approach of mathematically modeling networks and asking over what range of parameters a given behavior can be found (see Stelling et al., 2004 for review). Recent studies have modeled complicated networks (such as the networks controlling bacterial chemotaxis or those controlling segmentation in flies) and asked what fraction of parameters in the model will still support a given phenotype, with a common theme being that feedback loops allow a desired behavior to exist through a large fraction of "parameter space" (if it is imagined that mutation changes the parameters of the network, then the feedback in question makes the network genetically robust).

Experimentally, a treatment that increases the phenotypic variance of a trait in a genetically heterogeneous population has generally been considered to have compromised a mechanism for robustness. For example, Waddington found that treating a population of *Drosophila* larvae with elevated temperatures increases variation in several traits (Waddington, 1953). Moreover, the interindividual differences that appear after such a temperature treatment are selectable, and, once selected for, the phenotypes can become fixed (stabilized) even in the absence of heat stress. This suggests that the stress-induced increase in phenotypic variation in outbred lines is due to the uncovering of pre-existing genetic differences that did not result in phenotypic differences prior to the treatment (McLaren, 1999). Hence, the elevated temperature is argued to have compromised some as-yet-unknown genetic-robustness mechanism, thereby revealing previously hidden genetic variation.

Recently, it has been proposed that the temperature-responsive robustness factor in these particular experiments is the protein chaperone Hsp90. Several studies in a number of organisms have shown that genetic and pharmacological interference with Hsp90 function uncovers previously hidden selectable variation in multiple traits (Queitsch et al., 2002; Rutherford and Lindquist,

1998; Sollars et al., 2003). Hence, Hsp90 is argued to be a protein that canalizes several phenotypes and confers genetic robustness. The altered phenotypes become fixed even when Hsp90 function is restored, which indicates that they are heritable and therefore are unlikely to simply result from increased susceptibility to environmental noise. Interestingly, in one case, decreased Hsp90 activity leads to an increase in phenotypic variation even in nearly isogenic inbred lines (Sollars et al., 2003), which suggests that Hsp90 activity could have uncovered hidden *epigenetic* differences in the population. These studies, in aggregate, suggest that Hsp90 acts as a buffer that protects phenotypes against genetic mutations and/or epigenetic variation.

However, although it is irrefutable that loss of Hsp90 function indeed uncovers previously hidden genetic or epigenetic variation, an elegant theoretical study argues that this does not necessarily reflect a loss of true genetic robustness (defined as the insensitivity of a given phenotype to all possible mutations or, for quantitative phenotypes, a decrease in the mutational variance Vm; Hermisson and Wagner, 2004). Theoretical (Bergman and Siegal, 2003) and experimental studies (Mackay, 2001) show that hidden genetic variation is an intrinsic property of complex biological systems, and Hermisson and Wagner argue that most studies on robustness have used organisms that have been under selection, meaning that most alleles contributing to deleterious variation will be hidden. A change in conditions or certain mutations can then lead to the "release" of hidden genetic variation so that preexisting genetic differences between individuals in a population now result in visible phenotypic differences. The perceived role of Hsp90 in cellular robustness could therefore simply reflect its central, highly interconnected position in cellular networks. Mutations in *HSP90* or changes in its expression level therefore represent a dramatic change for the cell and result in the uncovering of some hidden variation. However, this does not mean that the assayed phenotype is now on average more sensitive to all possible mutations, as is required for a true mechanism of genetic robustness (for details, see Hermisson and Wagner, 2004).

Whatever the mechanism (conditionally hidden variability or true genetic robustness), it is clear that certain phenotypes are stabilized in the face of some genetic and epigenetic variation. Moreover, studies such as Waddington's indicate that in some cases, the environment can interfere with these systems and lead to an increase in heritable phenotypic variance. In other words, there appear to be mechanisms that regulate the timing of variability in response to the environment. Below we discuss further examples of such environmentally responsive mechanisms.

Mechanisms Regulating the Timing of Variation

Apart from regulating the timing of epigenetic switches and hide-and-release mechanisms (discussed below), some organisms may be able to vary the (global) genetic mutation rate. Specifically, when organisms experience stressful environments to which they are by definition poorly adapted, they often exhibit greatly increased phenotypic variation. This occurs by at least two mechanisms: (1) the uncovering of pre-existing hidden variation or (2) the generation of de novo variability, for example by increased mutation, transposon activity, or sex.

In 1988, John Cairns and coworkers found that when *E. coli* strains that carried an amber mutation in the *LacZ* gene were plated with lactose as the sole carbon source, a great number of Lac^+ mutants accumulated a few days later (Cairns et al., 1988). They concluded that starvation for a carbon source on lactose-containing medium activated a cryptic mechanism that allowed the cells to specifically direct mutation to the *LacZ* gene, thereby generating many more Lac^+ revertants than could be explained by spontaneous mutation alone. This theory of "directed mutagenesis" led to a fierce debate in the scientific community. When the dust settled, it appeared that Cairns's observations could be explained by an increase in the copy number of the (still partially functional) *LacZ* gene, which resulted in an increase in the absolute, but not relative, number of mutations (Andersson et al., 1998).

However, while Cairns's observations may be explained by alternative hypotheses, his work and the discussion it provoked made it clear that the Luria-Delbrück experiment had been historically overinterpreted, which was exactly Cairns's main point (Cairns et al., 1988; Rosenberg, 2001). The Luria-Delbrück experiment only investigated one specific type of mutation, so it did not exclude that other types of mutations could occur in a nonrandom fashion. More importantly, in the Luria-Delbrück experiment, the selective pressure is extremely severe and all nonresistant cells are killed in a short time, before they can acquire the necessary mutations and/or produce resistant offspring. In other words, the classic experiment only proves that at least some mutations take place before selection but does not prove that selective pressure is unable to stimulate additional mutations. When less severe selective stress was applied, it was found that in *E. coli*, sublethal stress may indeed influence the overall mutation rates in a process named "adaptive mutagenesis" (Bjedov et al., 2003; Hastings et al., 2004; Rosenberg, 2001).

In bacteria, several mechanisms exist by which stress can lead to an increase in DNA-sequence mutation (reviewed in Bjedov et al., 2003; Rosenberg, 2001). The best known mechanism for inducible mutagenesis is, arguably, the so-called SOS pathway in *E. coli* (Figure 4). Here, stressful conditions trigger the activation of special error-prone DNA replicases, which in turn leads to a massive increase in per-generation mutation rates.

Stress-induced mutagenesis is not limited to microbes. It was recently found that irradiation of male mice causes elevated mutation rates in the (nonexposed) first- and second-generation offspring (Barber et al., 2002). Although the mechanism behind this phenomenon is as yet unknown, trivial explanations (such as radiation-induced mutations in DNA-repair genes) have been ruled out due to the non-Mendelian inheritance of the phenotype and the lack of direct radiation exposure in some of the cells

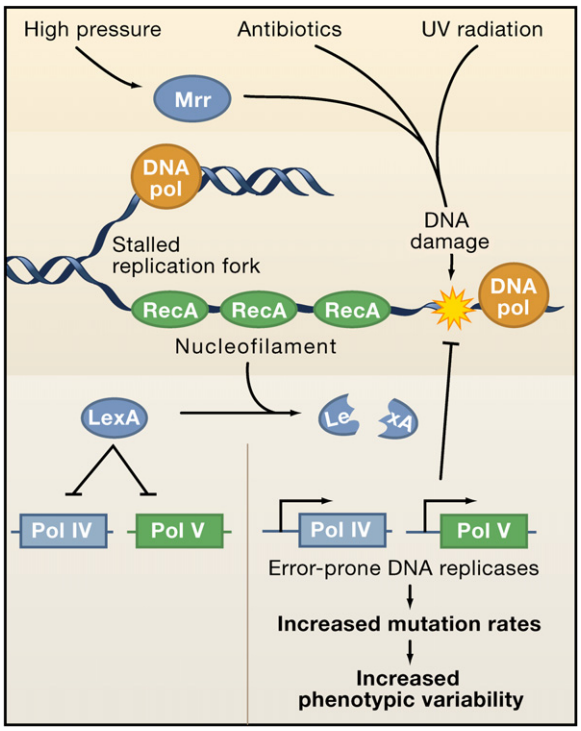

Figure 4. The SOS Pathway in *E. coli*

Various forms of DNA damage or perturbation lead to stalling and dissociation of the replication machinery. Single-stranded DNA is quickly stabilized by the RecA protein, and this nucleoprotein filament induces the autoproteolytic activity of LexA. Cleavage of LexA relieves repression of the 43 genes in the SOS regulon, which are involved in various DNA-repair processes. In particular, a special category of DNA polymerases is activated upon SOS induction. These polymerases can bypass irregularities at damaged sites in the DNA. However, they show error rates that are approximately 100-fold higher than those of normal DNA polymerases, thus earning them the name "error-prone polymerases" or "mutases." DNA damage is the best known inducer of the SOS pathway, but recent research shows that other forms of stress that are not directly related to DNA damage also activate an SOS response. These include starvation, exposure to antibiotics such as β-lactams, and exposure to physical stress such as elevated hydrostatic pressure (for a recent review, see Aertsen and Michiels, 2006). Although activation of the SOS pathway has been demonstrated for these cases, the exact trigger of the pathway remains unknown. It is possible that these stresses, through a yet-unknown mechanism, cause DNA damage that results in SOS activation. In the case of starvation, for example, it has been suggested that the lack of nutrients may result in the intracellular accumulation of DNA-damaging agents and the decrease of DNA-repair enzymes (Bjedov et al., 2003). Alternatively, the SOS pathway may also be triggered by more specialized stress-sensing mechanisms, as seems to be the case for β-lactam exposure, which depends on the two-component system DbiB/A, and for hydrostatic pressure, which relies on the MrrIV restriction endonuclease (Aertsen and Michiels, 2006).

that exhibit the phenotype. Instead, the mechanism appears to be more complex and may involve epigenetic alterations, as irradiation also causes a significant reduction in the levels of methyltransferases in (nonexposed) bystander tissue. (Barber et al., 2002; Koturbash et al., 2006). A similar phenomenon was recently described in *Arabidopsis*, where stress causes increased homologous recombination rates in at least four generations of the progeny of treated plants (Molinier et al., 2006).

Induced mutagenesis may not always rely on increased error rates during DNA replication. In the *Bacillus subtilis* "K-state" response, stationary-phase cells become competent after synthesizing specific complexes that mediate the uptake of foreign DNA (Hahn et al., 2005). It has been suggested that the K-state is required to provide a template for repairing damaged DNA that accumulates during stationary phase (Berka et al., 2002). However, depending on the DNA nearby, the uptake of foreign DNA could also increase genetic variability in stressed cells, and in human pathogens this is feared to enable rapid acquisition of antibiotic resistance (Prudhomme et al., 2006).

Yet another mechanism for increasing a population's genetic variability under stressful conditions is exhibited in organisms that increase the frequency of sexual reproduction under stressful conditions. Although the exact evolutionary benefit of sexual reproduction remains a topic of debate, certain experiments indicate that sex may indeed increase phenotypic variability in times of stress (for an example, see Greig et al., 1998). The choice of sexual, as opposed to asexual, reproductive strategies provides a species with a way to increase the variation in a population during hardship; individual organisms essentially gamble that their offspring will be more fit than they are due to a novel combination of alleles, and the species as a whole enjoys increased genetic variability.

Directed Mutagenesis Revisited?

In some cases, organisms seem able to change both the timing and focus (location) of phenotypic variability in response to the environment. For example, in *E. coli*, derepression of genes in response to nutritional stress appears to result in a specific increase in mutation rates of the coding sequences in question, apparently due to the exposure of single-stranded DNA during the transcriptional process (see Wright, 2004 for a review). Here, then, it appears that the organism's production of genetic variation is somewhat biased toward regions of the genome most likely to be involved in reducing the stressful situation. This, of course, is very similar to the suggestion by Cairns and coworkers noted above (Cairns et al., 1988), and it is unclear to us whether alternative explanations (such as the selective amplification of the target sequences that may explain Cairns's observations) could account for the phenomena described here. In any case, evidence is accumulating that some types of stress result in mutagenesis or recombination targeted to derepressed loci, which demonstrates environmental targeting of genetic variability (whatever the underlying mechanism).

In mammals, at least two mechanisms have been described that increase local mutation rates in response to environmental conditions. Perhaps the best-known system is "somatic hypermutation," where activated B cells express activation-induced cytidine deaminase, which results in an increase of six orders of magnitude in C → T

transitions (Honjo et al., 2005). Somatic hypermutation is largely (though not entirely) confined to the regions of antibodies that recognize antigens. Hence, somatic hypermutation is regulated both in locus (the antibody gene) and in time (during an infection to which the antibody is responding). The mechanism increases the diversity of antibodies on the sequence framework of a previously successful antibody, thus allowing the cell to locally explore sequence space in search of improved antigen-binding affinity. Although the biochemical mechanism for somatic hypermutation appears to restrict the mutagenesis to transcribed sequences, it is otherwise unclear how this activity is targeted. Somatic hypermutation is perhaps the clearest example of a physiological role for the environmental regulation of local phenotypic variation, although in this case the induced variation is only heritable in cell lineages within the organism and does not cross organismal generations.

A second system in mammals increases mutation rates over parasitic DNA elements such as transposons (Garrick et al., 1998). In addition to silencing these parasites, methylation of cytosine residues leads to accumulation of mutations in the relevant sequence because the deamination of methylcytosine (resulting, after replication, in a C → T transition) occurs an order of magnitude more rapidly than does the deamination of unmodified cytosine (2×10^{-7} per bp per generation as opposed to 2×10^{-8} per bp per generation for unmethylated cytosine; Garrick et al., 1998). Similar mechanisms have been intensively studied in the fungal kingdom. In *Neurospora crassa*, for example, repetitive DNA is inactivated by a DNA methylation-dependent process known as repeat-induced point mutation (RIP; Selker et al., 2003). It is therefore conceivable that directed methylation could provide organisms with another means to locally increase mutation rates at selected loci in response to their environment.

Localized Uncovering of Hidden Variation

Similar to directed mutagenesis, the hide-and-release or buffering mechanisms described above provide examples where variation at only a subset of genomic loci may respond to specific environmental conditions. Stress-induced decrease in Hsp90 function uncovers previously silent mutations in Hsp90 client proteins, which tend to be signaling molecules (Queitsch et al., 2002; Rutherford and Lindquist, 1998). Although it remains unclear whether Hsp90 represents a true robustness factor or a mechanism for the hide-and-release of phenotypic variability, the responsiveness of Hsp90 to environmental conditions allows organisms to uncover locus-specific phenotypic variation at stressful times. In other words, the fact that Hsp90 only interacts with a subset of proteins means that when Hsp90 levels vary due to environmental influences, only a specific set of phenotypes will increase their variance in the population.

In addition, many mechanisms of epigenetic inheritance described above not only make certain phenotypes more variable but also influence selected types of genetic variation and are responsive to the external environment. Specifically, subtelomeric genes are highly genetically variable in yeast, presumably because when silenced they are largely invisible to selection, while a similar argument may be made for highly variable 3′ untranslated regions that are not translated in the epigenetic [psi−] prion state of yeast. The subtelomeric silencing complex (described above) is inactivated by stress (via phosphorylation of Sir3), possibly allowing environmentally regulated uncovering of the subtelomeric genetic variation in a population (Ai et al., 2002). Similarly, the protein chaperone Hsp104 modulates the propagation of the [PSI+] prion state, and during heat and chemical stress it is observed that the [PSI+] phenotype is suppressed, presumably due to increased Hsp104 activity that releases functional Sup35 from prion aggregates (Eaglestone et al., 1999). Here again, stress-induced change in an epigenetic phenotype provides a mechanism by which the environment may influence the uncovering of hidden genetic variation (in 3′ UTRs), although in this case the seemingly paradoxical observation is that stress transiently decreases the readthrough phenotype of [PSI+] yeast. Both of these mechanisms thus provide regulatable bridges between epigenetic variation and genetic variation, which allows certain types of genetic variation to be uncovered in response to environmental regulation of epigenetic switches.

Regulated subtelomeric silencing and prion folding thus can be considered part of the hide-and-release class of mechanisms that allow hidden genetic variation to accumulate without phenotypic effect. Each of the hide-and-release mechanisms hides a particular type of genetic mutation in signaling genes (Hsp90 clients), in subtelomeric genes, or in 3′ UTRs, which results in regulatable release of localized variation. However, this releasable variation is expected to be largely random (except for its location). We now finally turn to the idea that organisms may orchestrate specific, nonrandom heritable changes in themselves in response to appropriate conditions.

Environmentally "Directed" Heritable Phenotypes?

We have outlined a number of mechanisms by which organisms modulate the timescale over which a phenotype is stable and mechanisms by which organisms increase seemingly random phenotypic diversity in response to stressful environments. Beyond this, organisms may not only randomly increase heritable variation in response to stress but in fact may inherit environmentally directed phenotypes in some cases, with the inherited phenotype being determined by the environment. The "inheritance of acquired phenotypes" is, of course, generally described as Lamarck's theory of evolution. In fact, Darwin also believed that the parental environment influenced progeny and incorporated some of Lamarck's basic ideas in his theory. However, the inheritance of acquired phenotypes was discredited by August Weismann (Weismann, 1893) and all but disappeared from the "New Synthesis," the modern theory of evolution that gradually developed during the first part of the 20th century and that has

dominated evolutionary science ever since. Nevertheless, as the next examples demonstrate, there is evidence for at least a few instances where the recent history of the organism indeed influences heritable traits, and we expect that more examples remain to be discovered.

Perhaps the clearest example of heritable consequences of environmental regulation comes from the fimbriae (*Fim*) genes in *E. coli* (Klemm, 1986). Similar to the phase-variation system in *Salmonella* (see above), the expression of the structural fimbriae gene *FimA* in *E. coli* is controlled by the orientation of a promoter element that is surrounded by inverted sequence repeats. Two proteins, FimB and FimE, play a role in catalyzing the recombination event that changes the orientation of this promoter (Klemm, 1986). FimB plays a role in both switches (on-to-off and vice versa), whereas FimE biases the switch in the on-to-off direction. Interestingly, temperature inversely regulates *FimB* and *FimE* with a net result of fimbriae expression at mammalian body temperatures (Olsen et al., 1998). Furthermore, *FimB* expression is repressed by extracellular sialic acid, which may provide a signal indicating elevated host defense mechanisms. Decreasing *FimB* expression ultimately results in decreased *FimA* expression and thus reduction of antigenic and proinflammatory fimbriae synthesis, which likely aids the organism in eluding the host immune system. Hence, the environment exerts an extremely localized, directed, and heritable effect on the genotype (promoter left or promoter right) of this microbe. This environmental regulation, although conceptually similar to the more common regulation of gene expression by transcription-factor control, clearly has distinctive characteristics due to the heritable nature of the induced phenotypes. Similar results hold in some other examples of phase variation; growth in different carbon sources or at different temperatures biases the equilibrium of the on-to-off switch of the *papBA* operon in *E. coli* (Blyn et al., 1989).

Plants, which do not segregate germline cells early in development, often appear to violate the "central dogma." Perhaps the most notable example is that of genomic rearrangement in flax. Here, changes in nutrient concentration can induce a reproducible programmed genomic rearrangement that includes changes in the copy number of rRNA genes and widespread, but reproducible, insertion events at certain loci distributed over all 15 flax chromosomes (Cullis, 1973; Henikoff, 2005). Although the underlying mechanisms and physiological consequences are not yet clear, this phenomenon suggests a directed, or "Lamarckian," change in the heritable material (Henikoff, 2005).

As we have described, metazoans can inherit a subset of DNA cytosine methylation patterns as well as some stable RNA molecules. Both cytosine methylation and RNA abundance are often regulated by external conditions, which makes it very easy to imagine mechanisms by which the environment may exert very specific transgenerational effects in metazoans. Indeed, a recent study in rats found that treating gestating mothers with the endocrine disrupter vinclozolin resulted in decreased male fertility in the progeny and that this phenotype was heritable for at least four generations (Anway et al., 2005). The authors suggest that the mechanism for this inducible inheritance was induced changes in cytosine methylation patterns, although genetic alterations to the Y chromosome were not ruled out. It is important to note that the study used a toxin as the environmental agent, and it is unclear what adaptive advantage would be served by inheritance of decreased fertility. Similarly, one can imagine that the offspring of animals treated with cytosine methylation inhibitors would also inherit a specific induced phenotype caused by the lack of appropriate imprinting during parental gametogenesis but that this "inducible" transgenerational phenotype is unlikely to have been selected for. Nonetheless, this study does provide an example of an environmental regime that exerts specific transgenerational effects in mammals.

The possibility of such transgenerational effects in mammals is of utmost importance to human disease and would have a profound impact on epidemiology. Fascinatingly, two recent epidemiological studies in human populations have implicated *ancestral* environments (food supply and smoking history) as potential risk factors for cardiovascular disease (Kaati et al., 2002; Pembrey et al., 2006).

Conclusion

Here, we have discussed mechanisms by which cells manipulate the location and/or timing of phenotypic variation. In many of these cases, it has been argued (or it may be argued by extension from other examples) that these cellular mechanisms tune the variability of a given phenotype to match the variability of the selective pressure that acts upon that phenotype. Phenotypes that are under swiftly changing variable selection are made more variable, whereas phenotypes under constant "stabilizing" selection are protected from variation by canalization, which results in their robustness. Other mechanisms seem to increase the phenotypic variability exactly when the selective pressure changes (i.e., in times of stress).

The analysis is complicated by the fact that, in most cases, we do not have a good idea of the selective pressure, and we realize that some examples above are extremely speculative. However, in at least some of the cases where the main selective pressure is understood (e.g., pathogens battling the host immune system) or where it is possible to set up controlled experiments, the evidence is mounting. Many of the most convincing examples that demonstrate a correlation between selective pressure and heritable variability come from the microbial world. This is partially due to the fact that laboratory studies are relatively easy in these organisms, so our knowledge of variability is simply greater for microbes. But there may be additional reasons. First, microbes tend to have much larger effective population sizes than do multicellular organisms, whose evolution is therefore more likely to be influenced by genetic drift. More interestingly, when

compared to cells in multicellular organisms where homeostatic mechanisms maintain relatively constant conditions for most cells, microbial cells are under severe and extremely variable selection. Microbes often find themselves subject to rapid environmental change (and thus variable selection) without any means of escape. They experience rapid changes in nutrient levels, osmolarity, concentration of (toxic) chemicals, and, in the case of pathogens, the continuous dynamic battle against host immune defense. Hence, it is perhaps not surprising to find that at least some microorganisms have developed mechanisms to maximize variability when and where it is most needed. Most of the described variability is in the cell surface, which is the cell's most direct interface with the environment. Notably, the best known example of regulated variability in higher eukaryotes is that of increased variability in the immune system (which interacts with highly variable pathogens).

At first sight, this close relation between variability and selective pressure contradicts today's Neo-Darwinian view on evolution. This is only partially true, as the examples do not argue against the randomness of the majority of phenotypic variability. However, the facts lead us to believe that selective pressure and phenotypic variability are not completely independent. It is easy to imagine how organisms may have developed mechanisms to influence their own phenotypic variability and escape the total randomness of "blind" mutations. Generating variability is a dangerous affair, with many changes leading to reduced, instead of improved, fitness. Hence, organisms that have developed methods to protect vital phenotypes for which abrupt changes in selection are unlikely while maximizing variability for phenotypes that have to respond to frequent variations in selective pressure may have had a selective advantage over individuals that did not have such systems. An analogous argument can be made for mechanisms that regulate the timing of variability.

It is interesting to note that in his book *The Origin of Species* Darwin wrote: "I have hitherto sometimes spoken as if the variations … were due to chance. This, of course, is a wholly incorrect expression, but it serves to acknowledge plainly our ignorance of the cause of each particular variation. [The facts] lead to the conclusion that variability is generally related to the conditions of life to which each species has been exposed during several successive generations." Hence, both Darwin and Lamarck, two of the founders of evolutionary theory, predicted that evolution itself may favor the development of self-guiding mechanisms, maximizing variability where and when it is most likely to yield positive changes while minimizing phenotypic variability when and where it is not needed. It is becoming increasingly difficult to argue that their general idea of nonrandom evolution was entirely wrong.

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