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Stress-Induced Modulators of Repeat Instability and Genome Evolution

Natalie C. Fonville[a] R. Matthew Ward[a] David Mittelman[a, b]

[a]Virginia Bioinformatics Institute and [b]Department of Biological Sciences, Virginia Tech, Blacksburg, Va., USA

Key Words
Microsatellite repeats · Genome instability · Stress-induced mutagenesis · Evolution

Abstract
Evolution hinges on the ability of organisms to adapt to their environment. A key regulator of adaptability is mutation rate, which must be balanced to maintain genome fidelity while permitting sufficient plasticity to cope with environmental changes. Multiple mechanisms govern an organism's mutation rate. Constitutive mechanisms include mutator alleles that drive global, permanent increases in mutation rates, but these changes are confined to the subpopulation that carries the mutator allele. Other mechanisms focus mutagenesis in time and space to improve the chances that adaptive mutations can spread through the population. For example, environmental stress can induce mechanisms that transiently relax the fidelity of DNA repair to bring about a temporary increase in mutation rates during times when an organism experiences a reduced fitness for its surroundings, as has been demonstrated for double-strand break repair in *Escherichia coli*. Still, other mechanisms control the spatial distribution of mutations by directing changes to especially mutable sequences in the genome. In eukaryotic cells, for example, the stress-sensitive chaperone Hsp90 can regulate the length of trinucleotide repeats to fine-tune gene function and can regulate the mobility of transposable elements to enable larger functional changes. Here, we review the regulation of mutation rate, with special emphasis on the roles of tandem repeats and environmental stress in genome evolution.

Introduction

Inherited variation is the raw material upon which natural selection acts, allowing organisms in a population to survive changes in their environment. Mutation rate is an important determinant of this variation and is likely the product of factors beyond the simple mechanical limitations of DNA repair proteins. The discovery of mutator and anti-mutator alleles, polymerases with different fidelities, and inducible error-prone repair suggests mechanisms for regulating mutation rate [reviewed in Caporale, 2003]. In addition, mutational hotspots can bias mutagenesis to specific sites such as double-strand breaks (DSBs). This review focuses on one type of mutational hotspot: tandem repeats, which are an important source of genetic variation in most organisms and have been demonstrated to facilitate adaptability through the modulation of gene function [reviewed in Gemayel et al., 2010].

The modern evolutionary synthesis suggests mutations are random with respect to their effect on fitness, and that there is independence between mutation and selection. Consequently, selection would only operate on genome variation that exists within a population prior to

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David Mittelman
Virginia Bioinformatics Institute
Virginia Tech, Washington Street MC 0477
Blacksburg, VA 24061 (USA)
Tel. +1 540 231 6430, E-Mail david.mittelman@vt.edu

the selection. This variation is proposed to accumulate at a constant rate, regardless of selective pressures and environmental stressors [Kimura, 1968]. These ideas have their genesis in August Weismann's proposal that somatic cells are derived from germline cells, and that while mutations in somatic cells could be expressed within an individual, these variations were not passed on to offspring. Conversely, he proposed that mutations in the germline, while not expressed in the individual, could be passed on to and expressed by an organism's offspring. This idea contradicted the Lamarckian theory that organisms adapted in direct response to their environment and subsequently passed those adaptations on to their progeny; therefore implying that mutation, environmental effects, and selection are separate phenomena [Kutschera and Niklas, 2004; Pigliucci, 2008].

This view was further supported by the classic fluctuation test experiments of Luria and Delbrück [1943]. This began with the observation that bacteria exposed to T1 bacteriophages occasionally displayed resistance to the phages. However, it was unclear whether this resistance was already present in the bacterial population, or arose in response to the selective pressure of the phages. When a number of independent bacterial colonies were plated, the number of phage-resistant mutants varied widely from colony to colony, suggesting that resistance-conferring mutations were already present in each population [Luria and Delbrück, 1943]. Thus, the experiment suggested that mutation and selection are separate processes.

However, the stress placed on the bacterial population in Delbrück and Luria's experiment was both sudden and extreme. Therefore, such an experiment might not comprehensively model all stresses facing evolving populations. Indeed, a growing body of evidence suggests that selection and mutation may not be entirely independent. For example, the offspring of male mice fed a low-protein diet exhibit elevated expression of genes for lipid and cholesterol biosynthesis in their livers, relative to offspring of males that receive a control diet. The altered gene expression is a result of epigenetic changes in the paternal mice, and therefore illustrates that a selection pressure in the environment could result in heritable changes to the genome [Carone et al., 2010]. Similarly, environmental stress increases the mutation rate in diverse species, including bacteria, yeast, and mammals [Cairns and Foster, 1991; Coyle and Kroll, 2008; Forche et al., 2011; Galhardo et al., 2007; McKenzie et al., 2000; Mittelman et al., 2010]. Such a connection between selection and mutation could even be evolutionarily advantageous, even if it was not evolved for that purpose, and provides a mechanism for organisms to rapidly adapt to environmental change [Galhardo et al., 2007; Mittelman et al., 2010; Rando and Verstrepen, 2007; Shee et al., 2011].

The ability of an organism to undergo mutation in response to environmental stresses suggests an ability to alter the *rate* of mutation, which could take effect globally or in specific genomic regions, and temporarily or permanently. Mutation rates could be altered globally and permanently by the presence of mutator alleles, temporarily due to transient events such as environmental stress, or locally at 'hotspot' locations in the genome (fig. 1). Certainly from an evolutionary perspective, it would be advantageous for mutation to be restricted in both time and space, since most functional mutations are likely to be deleterious. The focus of this review is to highlight findings on the effect of each of these factors on repeat mutation and genomic stability. Many tandem repeats are functionally important and can modulate morphological, behavioral and life history traits through quantitative effects on gene function. In addition, repeats are mutational hotspots and are further mutated by constitutive and stress-induced pathways of mutagenesis.

Repeats as Agents of Adaptability

Tandem repeats are dispersed throughout the genome, in and around gene regions. They are highly variable in most organisms and encode their own mutability through the unit size, length, and purity of the repeat tract [King and Kashi, 2007]. The high mutation rate of most repeat sequences led to initial assumptions that these sequences were 'junk DNA' or not functionally important. However, about 20 years ago, triplet repeats were identified as agents of disease. Since then, several microsatellite repeats (not all of which are triplets) have been identified as the underlying basis for a wide range of neurological and morphological disorders in humans and other mammals [Albrecht and Mundlos, 2005; Lopez Castel et al., 2010; Orr, 2009].

In addition to causing disease, microsatellites can exert subtle effects on gene function and quantitative traits. Coding microsatellites are enriched in transcription factors and other regulatory proteins, where changes in repeat length incrementally impact gene function [Albrecht et al., 2004; Gerber et al., 1994; Verstrepen et al., 2005]. Variations in the lengths of noncoding repeats in the promoters of genes have been shown to quantitatively affect transcription and can facilitate transcriptional plasticity [Vinces et al., 2009]. Emerging evidence impli-

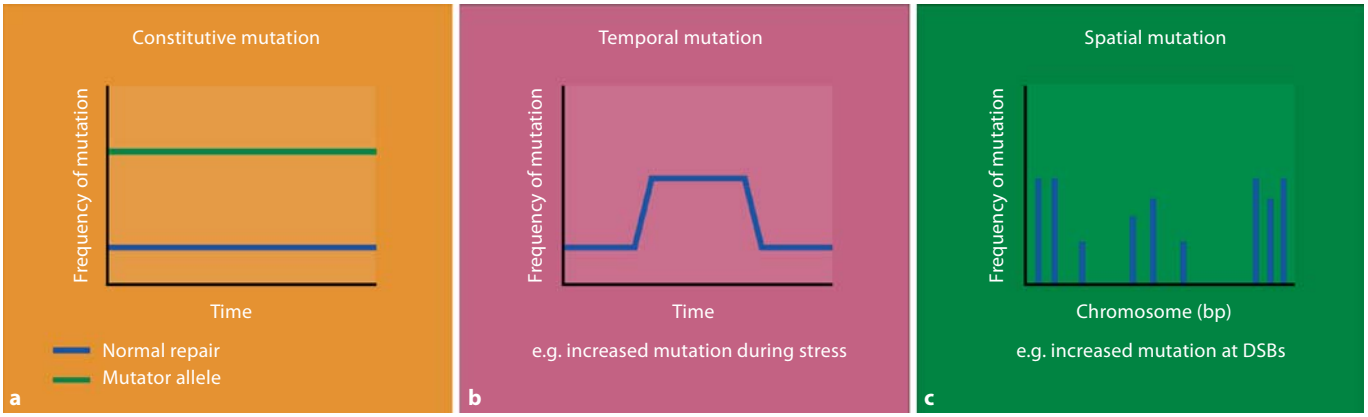

Fig. 1. DNA mutagenesis can be regulated constitutively, temporally, and spatially. Constitutive mechanisms (**a**) include mutator alleles that drive global, permanent increases in mutation rates. Other mechanisms focus mutagenesis in time (**b**) and space (**c**) to improve the chances producing adaptive mutations, while minimizing deleterious mutations.

cates coding and noncoding microsatellites as important sources of functional genetic variation in most species [reviewed in Gemayel et al., 2010]. Particularly in mammals, repeats have been shown to affect morphological and behavioral traits [Fondon and Garner, 2004; Hammock and Young, 2005].

Mutator Alleles

Several genes involved in DNA metabolism are essential, reflecting a critical requirement for maintaining DNA fidelity for fitness. Mutations in genes involved in DNA repair, such as those required for repair of mismatched bases, can increase the global mutation rate (fig. 1a). This is particularly relevant for asexual organisms where mutations that affect the global mutation rate (termed 'mutator alleles') can give rise to hypermutable organisms within a population [Whittam et al., 1998]. Many mutator alleles that have been identified are in the mismatch DNA repair (MMR) pathway, impairing the cell's ability to recognize and correct mispaired bases. The presence of mutator alleles can lead to decreased stringency when an organism undergoes homologous recombination (HR), because HR is highly dependent on MMR to prevent recombination between divergent DNA sequences [Rayssiguier et al., 1989; Surtees et al., 2004]. Therefore, impairment of MMR can lead to mutations that arise from misincorporated bases during DNA replication that remain unrepaired, or through genomic rearrangements caused by aberrant recombination.

The absence of recombination in asexual organisms enables mutator alleles to 'hitchhike' to high frequencies via linkage to any beneficial mutations they produce. In contrast, mutator alleles would not be expected to persist in sexual populations because recombination separates them from any beneficial mutations, and the ability to hitchhike is lost. However, in humans, mutator alleles have been described in somatic cells and are associated with certain cancers [reviewed in Loeb, 2001]. In particular, tandem repeats are destabilized by mutator alleles, and this is a major source of genetic instability in MLH1-deficient colon cancers [Bacon et al., 2000; Simpkins et al., 1999].

Stress-Induced Mutation

Although an increased global mutation rate could give rise to advantageous mutations, its utility is confined to asexual populations and the rare advantage mutations come at an incredible cost or mutational load. An alternative mechanism for increased mutation rate is stress-induced, or adaptive mutation which transiently upregulates mutation in response to environmental stress (fig. 1b). Although it occurs in multiple organisms, stress-induced mutation has been most clearly described in bacteria using the *lac*$^+$ frameshift reversion assay [Cairns and Foster, 1991], which has lead to the identification of two distinct mechanisms of adaptive mutation. First, after encountering a stressful environment (i.e. nutrient starvation), stationary phase cells (considered to be nondivid-

ing cells) can create an 'adaptive' frameshift reversion in an inactive lactose gene (a *lac*$^+$ frameshift reversion) allowing the cells to utilize lactose in the environment as a carbon source. A second mechanism occurs via amplification of a low-expressing gene to high copy number in order to produce a sufficient amount of the protein for growth. The amplified copies of the gene allowing adaptation are unstable and are easily lost from a culture in the absence of selective stress. Therefore, adaptive amplification represents a method by which bacteria can transiently adapt to a stressful environment and then rapidly return to their original state if the environment changes again.

Although the induction of an adaptive mutation is one possible outcome of stress, stressed cells also accumulate nonadaptive mutations at a higher rate than non-stressed cells, indicating that cells experiencing stress have an increased mutation rate genome-wide [Gonzalez et al., 2008; Torkelson et al., 1997]. This increase in mutation rate is not a general property of the cells that accumulate mutation. Instead, it is limited to times of stress, as shown by a requirement for the activation of at least three different stress responses: the cell envelope stress response [Gibson et al., 2010], the general stress response [Layton and Foster, 2003; Lombardo et al., 2004] and the SOS DNA damage stress response [McKenzie et al., 2000]. The mutation rate is increased 1,000-fold by the presence of a DSB in DNA [Ponder et al., 2005] and the mechanism underlying the increase in mutation rate appears to be a switch from high fidelity to error-prone repair of the DSB. The error prone polymerases DinB and Pol II are responsible for introducing point mutations during double-strand break repair (DSBR) in stressed cells [Frisch et al., 2010; Galhardo et al., 2009] because they are able to outcompete the non-error-prone polymerases at their stress-induced levels [Hastings et al., 2010]. Interestingly, recent work has indicated that the switch from high fidelity to error-prone DSBR is not necessary for the resolution of the DSB [Shee et al., 2011]. These results are very provocative in that they indicate stress-induced mutagenesis at DSBs is not simply a product of defective DNA repair. The opposition to the hypothesis of an optimized mutation rate has long been based on the argument that mutation is a 'mechanical inevitability', or the byproduct of physical limitations or defects in the fidelity of DNA repair processes, and not the product of natural selection.

In contrast to point mutation mechanisms in which polymerase errors are responsible for an increased mutation rate, adaptive amplification is hypothesized to occur via a transcription-coupled, microhomology-mediated, break-induced replication mechanism [Hastings et al., 2009a]. In humans, it is also hypothesized to underlie copy-number variation in humans [Hastings et al., 2009b]. Adaptive amplification is restricted temporally to times of stress, and through coupling mutation to transcription, amplification is also restricted spatially to those regions of the genome experiencing high transcriptional activity; the very regions that have the best potential for yielding an adaptive advantage. The ability to restrict mutations not only temporally but also spatially within the genome is critical for minimizing the accumulation of deleterious mutation, and is not unique to bacteria as multiple organisms have specific genomic regions that are more mutable and therefore classified as mutational hotspots (fig. 1c).

Repeats as Mutational Hotspots

Mutational hotspots are governed by a number of factors including the sequence and structure of the DNA itself [Wang et al., 2008]. Pathogenic bacteria have numerous hypervariable loci, termed contingency loci, which contain repetitive DNA elements and encode virulence factors critical for host-pathogen interaction. The hypervariability of these loci comes from slippage of the repetitive DNA, and creates variability in the population [Field et al., 1999; Moxon et al., 2006]. Tandem repeats are highly variable and prone to expansion and contraction mutations that result in the insertion or deletion of a repeated unit sequence. Several features of repeats including the purity and size of the repeated unit, as well as the length of the repeat tract, affect the mutation rate of repeats [Fondon et al., 1998; Legendre et al., 2007]. Microsatellite instability has also been shown to be induced by nearly any aspect of DNA metabolism including transcription [Lin and Wilson, 2007], methylation [Gorbunova et al., 2004], mismatch repair [Jaworski et al., 1995; Schweitzer and Livingston, 1997], nucleotide excision repair [Panigrahi et al., 2002] and base-excision repair [Kovtun et al., 2007]. Many of these processes have been proposed to affect repeat stability through 'correction' of slipped-strand structures that can arise during DNA metabolism [Parniewski et al., 2000]. Repeats are therefore mutational hotspots because the repair or even transcription of a repeat sequence is very likely to induce mutations in the sequence.

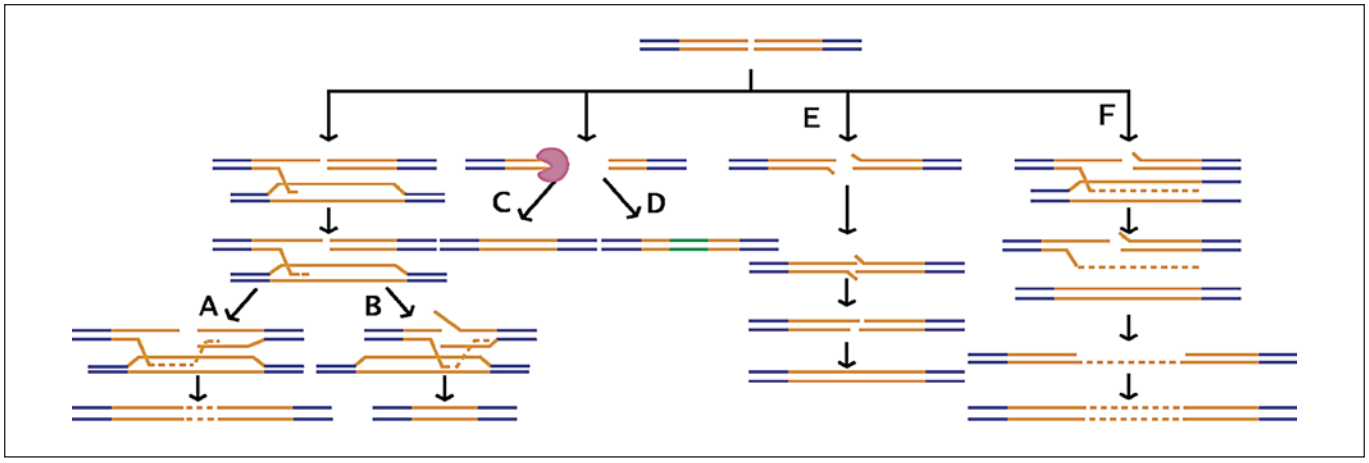

Fig. 2. Multiple DSBR pathways may contribute to the mutagenesis of tandem repeats. A: repair by HR with the insertion of repeat units; B: repair by HR with the deletion of repeat units; C: repair by NHEJ with a deletion of any number of bases that can disrupt the repeated unit pattern; D: repair by NHEJ with the insertion of nonrepeat bases; E: repair by SSA with the loss of a single or few repeat units; F: repair by synthesis-dependent strand annealing with the replication of additional repeat units. The orange lines indicate the repeat region, the blue lines indicate flanking nonrepetitive sequence, and the green lines indicate inserted nonrepeat sequence. The dashed orange lines indicate new synthesis of repeat region and the purple pac-man represents degradation of DNA during NHEJ.

DSBR in Repeat Regions

Repetitive DNA sequences are prone to DSBs [Cleary et al., 2002; Jankowski et al., 2000; Marcadier and Pearson, 2003; Nag and Kurst, 1997], and error-prone repair of these breaks is one mechanism by which repeats can mutate. DSBs in repeat regions can be repaired by diverse mechanisms including HR, nonhomologous end joining (NHEJ), and RAD51-independent strand annealing pathways (single-strand annealing, SSA; synthesis-dependent strand annealing) of the broken DNA ends (fig. 2). The majority of breaks are improperly repaired and result in the addition or loss of complete repeat units. But some breaks in repetitive DNA are repaired by insertion of nonrepeat bases by the mutation-prone DNA polymerase kappa in a way that disrupts the repeat [Hile and Eckert, 2008]. The exact contribution of DSBR pathways to repeat mutation is not yet clear, and is an area of active study. In one study, microsatellite repeats were destabilized by the impairment of RAD51 [Mittelman et al., 2010]. Since SSA increases in the absence of RAD51 [Mansour et al., 2008], it is likely that SSA contributes to the increased microsatellite instability in the absence of RAD51.

HSP90 and Stress

Genetic variation is easily described in terms of mutation and mutation rates. In contrast, phenotypic variation is easily visible, but is much more difficult to define because its sources can include multiple genetic and epigenetic factors. In eukaryotes, HSP90 is a molecular chaperone that functions to repair or activate proteins, but, in addition, can buffer genetic variation by forcing proteins to fold properly in spite of small changes to their sequence. During times of stress, such as heat shock, the number of proteins that require assistance in folding is increased, diverting HSP90 from its normal functions and revealing the cryptic genetic variation in the form of novel phenotypes. Titration of HSP90 during environmental stress is one proposed mechanism by which plants and animals can rapidly adapt to their environment.

Impairment of HSP90 also results in an increase in mutation rate of CAG repeats [Mittelman et al., 2010], suggesting an additional mechanism by which HSP90 can facilitate adaptation to stress: by mediating a switch from stable to unstable microsatellites. In bacteria, stress-mediated expansion of a triplet repeat resulted in a switch in the reductive function of a protein, possibly facilitating adaptation to oxidative stress [Ritz et al., 2001]. Although a beneficial role for stress-induced repeat instability has

Table 1. Hsp90 interacts with proteins involved in diverse pathways of DNA repair

Protein/complex	Role	Method of interaction	Reference
ATR	CHK1 activation and DNA damage response	direct	Ha et al. [2011]
BRCA2	HR of DSBs	direct	Noguchi et al. [2006] Dungey et al. [2009]
CHK1	cell cycle checkpoint and DNA damage response	indirect through ATR	Ha et al. [2011] Arlander et al. [2003]
DNA-PKcs/ERBB1	damage response to radiation	direct through ERBB1; contributes to HSP90 stability	Dote et al. [2006] Kang et al. [2008]
FANC complex	DNA crosslink repair	direct through FANCA	Oda et al. [2007]
MLH1	mismatch DNA repair	possible functional interaction	Fedier et al. [2005]
MRE11/RAD50/NBS1	MRN complex, repair of DSBs	direct through NBS1	Dote et al. [2006]
PIDD	p53-induced, NF-κB activation	Hsp90 binds cytoplasmic PIDD	Tinel et al. [2011]
Polymerase eta	translesion synthesis polymerase	direct; disrupts interaction with PCNA	Sekimoto et al. [2010]
RAD51	HR	indirect through BRCA2; possibly other interactions	Noguchi et al. [2006] Mittelman et al. [2010]
REV-1 polymerase	translesion synthesis polymerase	direct; disrupts interaction with PCNA	Mayca Pozo et al. [2011]
USP50/WEE1	cell cycle inhibition during DNA damage	direct through USP50	Aressy et al. [2010]

not been clearly demonstrated in eukaryotic cells, it is possible that this is one mechanism by which cells could facilitate adaptation to environmental stress. At minimum, Hsp90-induced repeat mutation might underlie some of the novel animal and plant phenotypes reported following Hsp90 impairment [Mittelman and Wilson, 2010]. The discovery that Hsp90 plays a role in the maintenance of genome suggests the environment can modulate genome variation, further connecting the forces of selection and mutation.

DSBR and HSP90

The HSP90 chaperone has now been demonstrated to affect many aspects of DNA repair and many of these interactions are summarized in table 1. HSP90 is a key component of HR, possibly through its regulation of BRCA2 folding. BRCA2 is an essential HR protein and mediates RAD51 filament formation [Jensen et al., 2010; Liu et al., 2010]. Inhibition of HSP90 has been shown to lead to altered RAD51 activity and also the degradation of BRCA2 [Noguchi et al., 2006]. Significantly, depletion of HSP90 and RAD51 using siRNA led to similar levels of repeat instability [Mittelman et al., 2010]. The increased instability in the absence of Rad51 likely indicates that breaks in repeats are normally repaired by HR in a conservative and faithful manner, but in the absence of RAD51, DSBR activity is altered allowing an alternative, error-prone pathway of repair. For example, in the presence of RAD51, strand invasion during HR may use a longer region for the homology search, having a greater chance for unique sequence flanking the repetitive DNA to be included in the alignment prior to recombination, while a shorter region of homology may lead to misalignment of repetitive DNA and expansion or contraction.

Although the role of RAD51 in HR at DNA breaks is well established, recent work has indicated that BRCA2 and RAD51 also play an important role in protecting stalled replication forks from degradation [Schlacher et al., 2011]. Replication forks may stall at secondary structures that form in repetitive DNA. The absence or alteration of RAD51 activity at these replication forks may allow for slippage structures to form, resulting in repeat instability.

Furthermore, the instability induced by HSP90 inhibition may not be completely due to HR and RAD51. HSP90 has been implicated in several different DNA repair path-

ways as it has been shown to interact with the MRN complex [Dote et al., 2006]. The MRN complex recruits both HR and NHEJ proteins to DNA breaks as well as regulates cell cycle proteins [Kim et al., 2005]. HSP90 has also been shown to interact with polymerase η [Sekimoto et al., 2010] and REV [Mayca Pozo et al., 2011], translesion synthesis polymerases which may contribute to repeat instability. The induction of repeat instability by HSP90 impairment or stress likely involves a switch to a more error-prone repair in at least the DSBR pathway. However, it is also possible that other stress-sensitive proteins regulate genome stability as well and that these stress-induced pathways might operate through multiple DSBR pathways as well as other repair pathways. This is supported by recent studies that show stress-induced mutagenesis in animals targets more than just tandem repeats.

Stress-Induced Transposon Activation

HSP90 has recently been shown to have a broader impact on genomic stability through regulation of the Piwi-interacting RNA pathway [Gangaraju et al., 2011; Specchia et al., 2010]. piRNAs are a special class of siRNAs whose main role is transposon silencing in the germline. In *E. coli*, stress-induced transposon activation can inactivate genes and activate otherwise cryptic operons, potentially providing an adaptive advantage to cells [Hall, 1999; Zhang and Saier, 2009]. Although most TE families are not mobile in human genomes, some long interspersed nuclear element 1 (LINE1 or L1) retrotransposons are still active. piRNA has been shown to silence L1 retrotransposition [Siomi et al., 2011]. Interestingly, transposons have been shown to integrate into repetitive DNA [Mancuso et al., 2010; Pan et al., 2010]. Retrotransposition of L1 into the genome may create insertions or deletions, affect gene expression, or alter the splicing of genes. Transposon integration into or excision from repeats might stimulate recombination, increasing microsatellite instability as well [discussed in Yant et al., 2005]. L1 mRNA is most commonly found in meiotic cells, but L1 insertions have been identified in neuronal progenitor cells, and have been shown to influence cell fate [Muotri et al., 2005]. It will be interesting in the future to examine whether activation of L1 or other transposons in neurons can be linked to microsatellite instability and/or neurological disorders. Microsatellite instability can impact social behavior [Hammock and Young, 2005], but whether such a case can be demonstrated for L1 retrotransposition remains to be seen.

Conclusion

Although there was an initial separation established between the forces of selection and mutation, emerging studies now suggest that the genome is more sensitive to the environment than previously suspected. The genome is dynamic and likely responsive to environmental stressors that might include heat, infection, inflammation, toxins, and changes in diet. There are many classes of mutation, all with different properties and rates of change; and some sites in the genome are more error prone than others (such as regions that accumulate DSBs).

Tandem repeats are an ideal model for studying the stability of the genome since they are mutational hotspots. Furthermore, repeat mutation is influenced by mutator alleles that cause variation on a genome-wide scale and is responsive to environmental stress. Importantly, repeats are also sources of functional variation upon which selection can act. The connection between HSP90 and microsatellites is particularly intriguing since it provides a possible mechanism to differentially mutate genes containing microsatellites. One area of future exploration will be to examine microsatellite instability on a genome-wide scale to determine the extent to which HSP90 inhibition induces changes to tandem repeats. The exact pathways that facilitate mutagenesis at repeats and other sites in the genome are of significant interest and will be an active area of research for the future as well.

Finally, repeats are likely just the first of many targets of stress-induced mutagenesis. So far, the additional targets include transposable elements and epigenetic patterns. Fortunately, fast and inexpensive sequencing methods such as next-generation sequencing technologies are poised to enable the comprehensive monitoring of genomes under stress. Next-generation sequencing has been used to document the genetic changes that underlie the transition from a normal cell to a malignant tumor cell [Ley et al., 2010; TCGA, 2008]. Future studies using whole-genome sequencing will dramatically enhance our understanding of stress-induced mutagenesis and reveal just how malleable the human genome really is.

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