

Stress-Induced Loss of Heterozygosity in *Candida*: a Possible Missing Link in the Ability to Evolve

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ABSTRACT Diploid organisms are buffered against the effects of mutations by carrying two sets of each gene, which allows compensation if one is mutated. But recombination between “mom” and “dad” chromosomes causes loss of heterozygosity (LOH), stretches of “mom-only” or “dad-only” DNA sequence, suddenly revealing effects of mutations accumulated in entire chromosome arms. LOH creates new phenotypes from old mutations, drives cancer development and evolution, and, in a new study by Forche et al., is shown to be induced by stress in *Candida albicans* [Forche A, et al, *mBio* 2(4):e00129-11, 2011]. Stress-induced LOH could speed evolution of *Candida* specifically when it is poorly adapted to its environment. Moreover, the findings may provide a missing link between recombination-dependent mutagenesis in bacteria and yeast, suggesting that both might be stress induced, both maximizing genetic variation when populations could benefit most from diversity.

Evolution is a two-way pull between the generation of variation in types of individuals in populations and preferential proliferation (“selection”) of those variants best suited to the environment. This feedback between genome (ultimately mutation) and environment drives cancer proliferation, pathogen adaptation to host defenses, and antibiotic and cancer chemotherapy resistance, in addition to the origin of species.

In contrast with initial assumptions that mutations accumulate constantly, gradually, and independently of selective environments (1), microbial and other geneticists have discovered stress-inducible mutagenesis mechanisms in bacteria, yeast, and human cancer cells (2). These mechanisms increase the mutation rate specifically when cells are maladapted to their environment, that is, when they are stressed, usually via the coupling of a mutation-generating pathway to one or more normal cellular stress responses. Stress-inducible mutation mechanisms increase genetic variation and potentially the ability to evolve and do so in a manner responsive to changing environments. Various stress-inducible mutation mechanisms produce point mutations, transpositions, gene amplifications, and copy number variations (2). In a recent issue of *mBio*, Forche et al. (3) describe a new twist on this theme. They show that in the pathogenic yeast *Candida albicans*, loss of heterozygosity (LOH) is also induced by stress.

LOH is often the pivotal event in generation of variation in diploid cells. Because diploids have two copies of each gene and because most mutations are recessive, new mutations usually have little or no effect. The other chromosome masks their phenotypes. During LOH, one chromosome, e.g., the “mom” chromosome, becomes identical to the other, e.g., the “dad,” often over long stretches, unmasking the phenotypes of previously acquired mutations in that region. LOH famously underlies the multihit nature of cancer development (4), the first “hit” being mutation and the second LOH (5).

A frequent cause of LOH is somatic homologous recombination (HR) used to repair DNA double-strand breaks and ends (DSBs/DSEs) (Fig. 1). Three kinds of LOH result from different HR/DSE repair events: LOH can occur in short chromosomal tracts by a gene conversion-like process (not shown) and in long tracts, including whole chromosome arms, by reciprocal recombination (crossover) or by “break-induced replication” (BIR).

Crossover between replicated “mom” and “dad” chromosomes generates LOH because, in somatic cells, the recombined chromatids segregate randomly such that a recombined chromatid can end up in a cell with a wholly mom or wholly dad homologue (Fig. 1A). BIR is a mechanism that repairs a single double-strand DNA end (DSE) that forms when a replication fork breaks or “collapses” (Fig. 1B) (6). In baker’s yeast, the DSE usually finds the chromosome it broke from, pairs with it, and may copy the entire length of DNA from the DSE to the telomere. This is usually a genetically silent event, e.g., a mom DNA end copying the mom chromosome—but occasionally a mom DNA end pairs with the dad chromosome and then can copy very long tracts of dad sequence, from the DSE to the telomere, producing LOH (Fig. 1B). LOH can also be caused by whole-chromosome loss, presumably by failure of segregation.

Forche et al. used elegant genetic assays to distinguish whole-chromosome loss (non-HR) and short- and long-tract (HR) LOH events at very many different genomic sites in different genetically marked reporter strains (3). They report that overall about 86% of spontaneous LOH events in *Candida* are long tract (HR/DSE repair type), 9% are short tract (HR/DSE repair type), and 5% are whole-chromosome loss (not HR). HR rules the day. Among the long-tract events at one locus, they used a sophisticated assay to capture the fates of all four chromatids after LOH and showed that most are of the nonreciprocal BIR type (Fig. 1B), which results from repair of a single DSE.

The authors then applied three different host-relevant stressing treatments to six of the reporter strains and showed that LOH events are increased dramatically and dose dependently with the stressors. The LOH events were measured by loss of a moveable *URA3* gene unrelated to the stresses applied. The stressors were

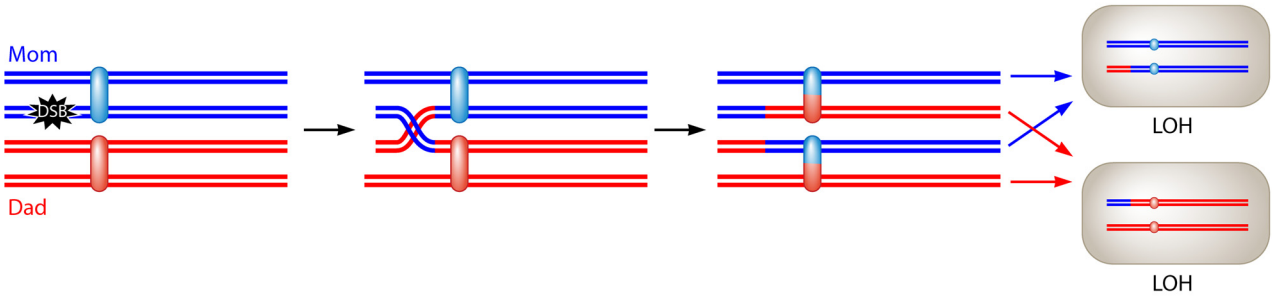
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A. LOH by Reciprocal Recombination



B. LOH by Break-Induced Replication

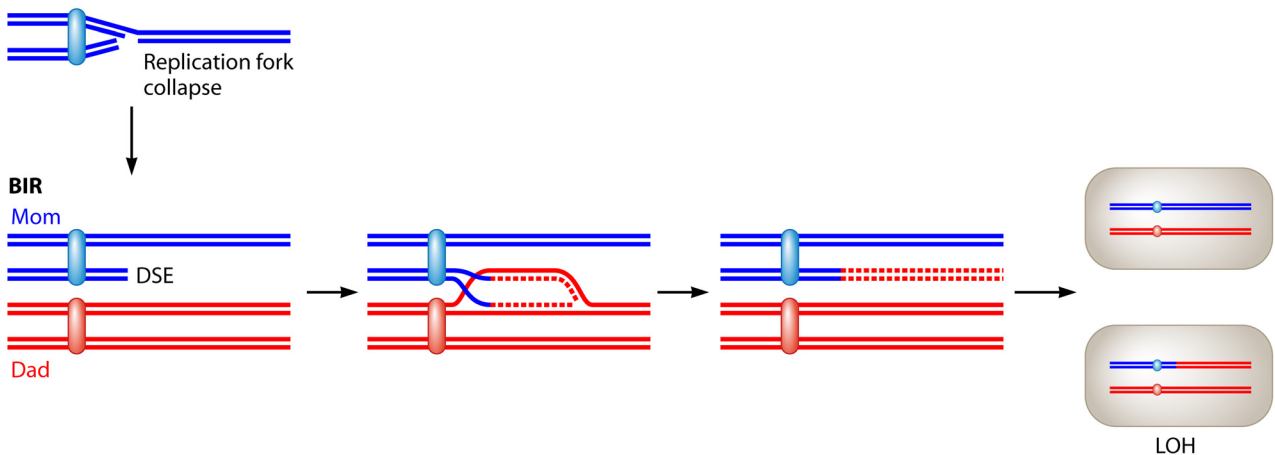


FIG 1 Recombinational routes to long-tract loss of heterozygosity. Lines represent strands of DNA; dashed lines indicate newly synthesized DNA; ovals indicate centromeres; “Mom” indicates one homologue, and “Dad” indicates the other. (A) In somatic cells, reciprocal recombination between replicated homologous chromosomes causes loss of heterozygosity when recombined chromosomes segregate with unrecombined ones. Because segregation is random in somatic cells, this is frequent. (B) Break-induced replication, or BIR, can cause loss of heterozygosity. Although the same chromosome is usually used in BIR repair of a collapsed replication fork (mom here; not shown), occasional use of the other (dad) chromosome can cause LOH.

heat stress, similar to what the organism encounters in a patient with fever; oxidative stress, as is launched by host immune cells; and the antifungal drug fluconazole, an ergosterol biosynthesis inhibitor used to treat *Candida* infections. Whereas heat increased LOH 1- to 40-fold (an uptick but not statistically significant), the oxidizing agent hydrogen peroxide (H₂O₂) increased LOH a statistically significant 3- to 72-fold, and fluconazole increased it a significant 285-fold. These stressors increased LOH even at doses that did not inhibit growth. This finding, and their finding of increased LOH at many different unrelated genomic sites, indicates that LOH events were induced by the stress, not merely selected as better survivors of a particular stress. Their results demonstrate stress-inducible loss of heterozygosity, which decreases genetic complexity of an individual but increases phenotypic diversity of the population. Though often deleterious, LOH can confer adaptive/proliferative outcomes (7), including, as noted, cancer development (5). Thus, *Candida* may have an enhanced ability to evolve specifically in an environmental/adaptive tight spot when it is stressed.

Each stressor increased some LOH types more than other types, indicating that the different stressors stimulated different mechanisms of LOH. H₂O₂, which damages DNA and proteins, increased short-tract and long-tract LOH, implying that HR, most

probably DSE repair, was increased. H₂O₂ could have caused DSEs directly or might alter the cellular enzymatic milieu via induction of stress responses, and these might promote HR and BIR. Heat increased whole-chromosome loss, possibly perturbing chromosome segregation machinery. Fluconazole increased both whole-chromosome loss and long-tract LOH. Whether these stressors increased LOH events via activating stress responses that upregulate LOH-generating pathways is not known. Identification of stress responses that might control LOH-promoting mechanisms is an intriguing area for further exploration.

There are obvious and less-obvious parallels between stress-induced LOH in *Candida* (3) and stress-induced mutagenesis mechanisms observed in bacteria, yeasts, and human cells (2). Obviously, regardless of how or why they evolved, both will generate variation in populations specifically when they are maladapted to their environments, that is, when cells become stressed. This paradigm, in which generation of variation can be environmentally responsive and variations temporally clustered (8–10), differs fundamentally from previous purely probabilistic models in which mutations and phenotype variations occur constantly and gradually over time, uncorrelated with each other or environmental input (1). See reference 11 for discussion of the environmentally responsive model, previous arguments against it, and

recent work that supports it, and see reference 2 for a more extensive but less current review.

Less obviously, the results obtained by Forche et al. (3) suggest a possible parallel with the best-understood stress-induced mutagenesis mechanism and a solution to a previous enigma. DSB-dependent mutagenesis was discovered in *Escherichia coli* as a stress-associated pathway (12, 13), then demonstrated and elaborated in baker's yeast (14–18), where it appeared to be stress independent. In both organisms, DNA polymerase errors made during acts of DSE repair via HR persist and become mutations. In *E. coli*, DSE repair synthesis is high fidelity (non-mutagenic) in unstressed cells but switches to a mutagenic mode, using specialized error-prone DNA polymerases, DinB, Pol V (19), and Pol II (20), during stress that activates the RpoS-controlled general stress response or if the RpoS transcriptional activator of the response is upregulated artificially in unstressed cells (11, 21). That is, the general stress response throws a switch that licenses the use of error-prone DNA polymerases in DSE/DSB repair, promoting mutations under stress, when cells are poorly adapted to their environment (11, 21). This switch is not needed for the repair itself (11, 21) and might be an evolution-enhancing mechanism. In baker's yeast, however, essentially all acts of DSB repair via HR seem to be mutagenic independently of any known stressor other than the DSB. Moreover, the kinds of DSE repair events that provoke DSB-dependent mutagenesis in baker's yeast are the same as those that induce LOH: gene conversions (14, 16, 17), probably reciprocal recombination, and BIR (18), and they do so using either a specialized error-prone DNA polymerase (15) or a housekeeping DNA polymerase(s) (17, 18). Hence, yeast DSB/HR-dependent mutagenesis was not known to be stress inducible.

The results obtained by Forche et al. (3) suggest that DSB-dependent mutagenesis may be stress inducible in both bacteria and eukaryotes. They show that LOH caused by HR (probably DSE repair) is stress inducible, implying that DSEs and/or HR is stress inducible. Thus, DSE/HR-dependent mutagenesis seems likely to be stress inducible in yeast, as it is in *E. coli*, albeit with the stress inducibility controlled at a different step in the mechanism. If baker's yeast is like *Candida*, then either DSEs or their apparently constitutively mutagenic repair would be expected to be stress inducible, whereas in *E. coli*, repair is constitutive but its mutagenicity is stress induced, controlled by RpoS (11, 21). These apparently separate evolutions of mutagenic DSE repair/HR may converge on a similar biological outcome, stress-inducible mutagenesis-associated DSE repair. Linking mutagenesis to DSE repair in a stress-inducible process could provide two evolution accelerators: the ability to make mutations specifically when maladapted and the ability to make them locally in DSB repair zones, which could promote concerted evolution (multiple changes) within genes and linked genes (21, 22), a significant limiting factor in protein evolution.

The work of Forche et al. (3) introduces a new dimension to stress-induced generation of variation, which can potentially fuel evolution specifically when populations are poorly adapted to their environments: stress-induced diversification by LOH. This adds to stress-induced mutagenesis (2) and stress-induced generation of phenotypic diversity by the unmasking of protein diversity during stress when chaperones become less available and by other protein-based mechanisms (23). The authors note also that

previous observation of aging-induced LOH in yeast (24) may reflect accumulation of stressors in aging cells and stress-induced LOH. Their work begs many intriguing questions, including what role, if any, stress responses play in the LOH mechanisms induced, which specific mechanisms underlie each specific LOH type observed, and how stress or stress responses promote them.

The two-way pull between genotype and environment that drives evolution appears to include feedback and responsiveness. The concepts of feedback and responsiveness were absent at the dawn of our understanding of genetic mechanisms underlying evolution (1), which, after all, predated molecular biology. But these concepts make sense to students of biological mechanisms and their control. Discerning underlying molecular mechanisms revealed this fluid, responsive view of mutagenesis and protein diversity (2, 23). The underlying mechanisms will be eagerly awaited for LOH in the future.

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