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Systemic and rapid restructuring of the genome: a new perspective on punctuated equilibrium

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Abstract

The rates and patterns by which cells acquire mutations profoundly shape their evolutionary trajectories and phenotypic potential. Conventional models maintain that mutations are independently acquired over many successive generations. Yet, recent evidence suggests that cells can also experience mutagenic processes that drive *rapid genome evolution.* One such process manifests as *punctuated bursts of genomic instability*, in which multiple new mutations are acquired simultaneously during transient episodes of genomic instability. This mutational mode is reminiscent of the theory of punctuated equilibrium, proposed by Stephen Jay Gould and Niles Eldredge in 1972 to explain the burst-like appearance of new species in the fossil record. In this review, we survey the dominant and emerging theories of eukaryotic genome evolution with a particular focus on the growing body of work that substantiates the existence and importance of punctuated bursts of genomic instability. In addition, we summarize and discuss two recent studies from our own group, the results of which indicate that punctuated bursts systemic genomic instability (SGI) can rapidly reconfigure the structure of the diploid genome of *Saccharomyces cerevisiae*.

Keywords

Genomic Instability; Aneuploidy; LOH; Mitotic recombination; Punctuated evolution; Genome evolution

Paradigms of evolution in the genomics era

Genomic mutations are essential for the evolution of biological systems. In the *Origin of Species*, Charles Darwin wrote: *'As natural selection acts solely by accumulating slight,*

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successive, favorable variations, it can produce no great or sudden modification; it can act only by very short and slow steps' (Darwin 1859). Drawing from the concept of *'Natura non facit saltum'* (*i.e.*, 'Nature makes no leaps'), Darwin portrayed evolution as an iterative machine powered by rare, independently acquired mutations and directed by the pressures of natural selection. Gradualism has become a central tenet of modern biology and remains a dominant lens through which biologists from diverse fields interpret genotypic and phenotypic change. Yet, gradualism fails to explain some evolutionary processes, particularly those that occur at seemly accelerated tempos. Perhaps the best example of this apparent 'accelerated' evolution is seen in the development of cancer. By the time they are detected, many cancer cells harbor remarkably complex patterns of chromosomal and karyotypic structural alterations (Alexandrov, et al. 2013, Garraway and Lander 2013, Kandoth, et al. 2013, Lawrence, et al. 2013, Vogelstein, et al. 2013, Zhang and Pellman 2015). Given that the known rates of these classes of mutation are low, it has remained unclear how cancer cells accumulate so many mutations so rapidly by a gradual mode of evolution alone. One alternative model, known as the mutator or hypermutation phenotype, suggests that some cancer cells can proliferate in a constant state of genomic instability, and as a result, acquire new mutations at elevated rates (Fig. 1). The hypermutation phenotype has been observed in cells harboring deleterious mutations which lower the fidelity of DNA polymerases or the activity of DNA repair proteins (Loeb 2016, Loeb, et al. 1974). This accelerated tempo also manifests as the phenotype known as chromosomal instability (CIN), a condition defined by constitutively increased rates of whole chromosome mis-segregation and loss-of-heterozygosity (LOH)(Weaverand Cleveland 2008). Another emerging model, which we refer to as 'saltational bursts' or 'punctuated bursts', proposes that cancer cells may acquire multiple mutations simultaneously during short-lived episodes of genomic instability (Fig. 1). This model derives from the theory of punctuated equilibrium, originally proposed by Niles Eldredge and Stephen Jay Gould in 1972 (Eldredge and Gould 1972), and describes a biphasic mode of evolution consisting of long periods of genome stability (*i.e.*, stasis) punctuated by short bursts of destabilization during which many genomic changes are rapidly acquired.

The advent of modern whole genome sequencing (WGS) technologies and bioinformatic analysis has enabled researchers to begin investigating the extent to which these mutational modes actually contribute to the evolution of genomes. Since then, numerous studies have provided compelling data suggesting that genomes can become dramatically restructured by bursts of mutation. Such bursts have been postulated to underlie types of genome evolution ranging from the expansion of gene duplications (Jiang, et al. 2007), to the activity of transposable elements (Oliver and Greene 2009). Additionally, a growing body of work supports the idea that punctuated genome evolution drives clonal expansion of human cancers (Cross, et al. 2016, Markowetz 2016). Indeed, numerous independent studies that analyzed the mutational landscape of breast, melanoma, and colorectal tumors using WGS analysis discovered that these tumors were composed of several sub-clonal populations that likely arose rapidly early in tumor initiation and stably expanded as the tumor grew (Casasent, et al. 2018, Cross, et al. 2018, Field, et al. 2018, Gao, et al. 2016, Gerstung, et al. 2020, Sottoriva, et al. 2015, Stepanenko, et al. 2015). Such population structures indicate that these tumors experienced a punctuated burst of genomic instability and were not driven

by a hypermutation phenotype, which would result in a tumor consisting of a highly heterogeneous population of genetically distinct cells. These studies, together with broader surveys of cancer genome evolution indicate that bursts could be a feature common to the development of many cancer types (for an excellent review on the topic, we recommend (Cross, et al. 2016)). Despite these advances, deducing the mutational modes at play in cancer genome evolution remains complicated by the fact that the genome of a tumor sample is typically characterized long after neoplastic initiation. As such, it is difficult to rule out the degrees to which modes like bursts may have contributed to the current mutational landscape of the genome. Thus, although punctuated bursts appear to significantly contribute to the evolution of diverse biological systems, critical outstanding questions remain unanswered: What types of mutations arise in bursts? On what time scale do bursts occur? What molecular and cellular events cause these bursts of genomic instability? How do these events contribute to the phenotypic plasticity, adaptive potential, and long-term evolution of the cell?

Using budding yeast to study the tempos of structural genome evolution

This year, our group published two parallel studies in which we used diploid *Saccharomyces cerevisiae* cells to define the tempos with which two classes of structural alterations arise in the genome (Heasley, et al. 2020, Sampaio, et al. 2020). In Sampaio et al., 2020 we investigated the patterns by which cells acquire LOH resulting from mitotic recombination, and in Heasley et al., 2020, we investigated the patterns by which cells acquire whole chromosome copy number alterations (CCNAs)(e.g., aneuploidies). Our results, summarized and discussed below, suggested that these large-scale alterations often arise in a burst-like pattern, and that these bursts can rapidly and dramatically alter the structure and content of the diploid genome.

In our studies, we took advantage of several strengths of the budding yeast model system to comprehensively asses the tempos with which LOH and CCNAs arise in the diploid genome: 1) The ability to recover clones harboring specific mutations using counter-selectable selection, 2) The ability to conduct quantitatively rigorous mutational analyses using small clonal populations (≤35 generations), and 3) the ability to inexpensively sequence the genomes of numerous clones with deep coverage. Operating under the conventional Darwinian premise that genomic alterations are acquired independently and gradually over many generations, we predicted that the rate at which a cell acquires two defined mutations (*e.g.*, $rate^{A+B}$) should be the multiplicative product of the rates at which each individual mutation occurs ($rate^{A+B} = rate^{A} \times rate^{B}$). We constructed diploid strains from which clones harboring defined structural alterations at two distinct loci in the genome could be selected. With these, we grew cells in normal conditions for fewer than 35 generations and used fluctuation analysis to determine the rates at which each individual mutation occurred as well as the rate at which both mutations arose in the same cell. For our analysis of the tempos by which *de novo* LOH occurs, we inserted hemizygous copies of the counter-selectable markers *URA3* and *CAN1* at genomic loci on chromosome IV (Chr4), Chr5, and Chr13 to create a suite of strains that could be selected for individual LOH events on Chr4, Chr5, or Chr13, as well as pairs of LOH events on Chr4 and Chr5, and Chr5 and Chr13 by plating cultures to media containing 5-fluoroorotic acid (5-FOA)(Boeke, et al.

1984), canavanine (Larimer, et al. 1978), or a combination of both drugs. With the rates derived from these experiments, we compared the 'predicted' rates at which a cell would be expected to acquire both tracts of LOH independently to the 'observed' rates at which cells harboring both LOH events actually appeared in a population. Intriguingly, cells harboring two selected tracts of LOH arose at rates 15- to 150-fold higher than predicted by the conventional model of mutation acquisition.

This higher-than-predicted incidence of double mutants was even more pronounced in our parallel study which examined patterns of coincident aneuploidization (Heasley, et al. 2020). We engineered diploid strains with two copies each of either the *URA3, CAN1*, or *TRP1* markers inserted on either side of the centromere of a specific chromosome such that we could select for different combinations of aneuploidies (loss of: Chr1 and Chr3, Chr1 and Chr5, Chr3 and Chr5, Chr9 and Chr5, Chr12 and Chr5). We found that aneuploidies of each individual chromosome occurred at rates ranging between $1.2x10^{-6}$-$2.1x10^{-8}$ events/cell/generation. Per Darwinian principles, cells harboring any of the above pairs of aneuploidies would be predicted to arise at the exceedingly low rates of 10^{-12}–10^{-14} events/cell/division. Remarkably, and in contrast to this prediction, we recovered cells harboring two aneuploidies at rates 600-fold to 3800-fold higher than expected. Together, results from our quantitative approach indicated that cells harboring multiple structural mutations arose more often than could be explained by a gradual model of genome evolution.

We independently validated the quantitative results of the selection assays described above by sequencing the genomes of derivative clones harboring one or two selected structural mutations. Using WGS analysis, we were able to descriptively assess the structure of these genomes, as well as to detect unselected mutations that co-occurred with the primary selected events. From this analysis, we found that unselected mutations frequently accompanied a primary selected event (Fig. 2). Whereas control clones isolated without any selection were free of structural alterations, 15.5% of clones selected for LOH possessed additional unselected tracts of LOH elsewhere in the genome and 44% of clones selected for aneuploidy harbored additional unselected aneuploidies. The read coverage depths deduced from our WGS analysis demonstrated that the majority of these unselected mutations had not accumulated after selection of the primary mutation and instead suggested that they arose during the same temporally restricted episode of genomic instability that had resulted in the acquisition of the primary selected mutation(s). Moreover, this result also indicated that, rather than showing signs of continued instability (*i.e.*, a mutator phenotype), most clones had stably propagated these newly reconfigured genomes throughout the growth of the colony that formed on the selective media plate.

Tempos and modes of genome evolution reconsidered

Together with the work of others (Forche, et al. 2011, Forche, et al. 2018, Hickman, et al. 2015), our studies demonstrate that cells can gain multiple mutations non-independently during very short-lived episodes of systemic genomic instability (SGI). In contrast with the principles of gradualism, it appears that sometimes, nature does make leaps. How do we reconcile this finding with established models of mutagenesis and genomic instability? Reports documenting the coincident acquisition of disparate mutations in the yeast genome

date back nearly 60 years (Fogel and Hurst 1963, Freeman and Hoffmann 2007, Golin and Esposito 1984, Golin and Tampe 1988, Wood 1982). We suspect that the same fundamental mode of punctuated SGI was responsible for those observations, but could not be fully appreciated and characterized without the benefit of modern whole-genome analysis. Selectable assays, such as the those used in our studies, have been a primary methodology with which to define various metrics of genome stability (*e.g.*, mutation rates)(Klein, et al. 2019, Putnam and Kolodner 2017). Yet, the historic and preferential use of highly homozygous isogenic laboratory strains for such assays has limited our ability to determine how the structure and composition of the entire genome changes when a specific selectable mutation is acquired. Because isogenic strains lack genetic markers throughout in the genome, the selectable mutation was likely the only genomic change detectable in past assays, resulting in the common assumption that it was the only mutation acquired in the genome of the selected cell (Heasley, et al. 2021). In our studies, we used highly heterozygous diploid strains so as to be able to interrogate greater than 25,000 loci distributed across the genome by WGS analysis. In doing so, we were able to detect other unselected and unrelated mutations that arose elsewhere in the genome coincident with a selected mutation.

The remarkably altered genomes characterized in our studies bring to mind the ‘hopeful monsters’ described in Richard Goldschmidt’s theory of macromutation in which he proposed that large-scale mutations (*e.g.*, chromosomal rearrangements) were likely to drive the large-scale evolutionary events that could not be explained by Darwinian models of gradualism (Dietrich 2000, Goldschmidt 1940, Wright 1941). As he wrote in *The Material Basis of Evolution* ‘A single mutational step affecting the right process at the right moment can accomplish everything…’(Goldschmidt 1940). He used the term ‘hopeful monsters’ to refer to individuals that had acquired such alterations because while most would likely suffer tremendous fitness deficits, a rare ‘monster’ with dramatic genotypic and phenotypic changes might survive and define a new species. Indeed punctuated bursts of SGI appear to be capable of producing a spectrum of ‘hopeful monsters’, a number of which do display new phenotypic variations. While Goldschmidt’s application of this premise to the complex and variable processes underlying speciation was in some ways flawed (Wright 1941), it remains tempting to speculate that bursts of SGI could produce novel phenotypes that would be unlikely to appear by gradualism alone. Moving forward, we are broadening our investigations of punctuated bursts to define how these events impact the phenotypic variation and adaptive potential of the cells which experience them.

What cellular events might cause these punctuated bursts of mutation? While numerous possibilities exist, we speculate that these events may occur when the activity of cellular processes such as DNA damage repair (Craven, et al. 2002), replication (Wilhelm, et al. 2019), sister chromatid cohesion (Covo, et al. 2014, Daum, et al. 2011), spindle assembly (Maiato and Logarinho 2014, Mattiuzzo, et al. 2011), and mitotic checkpoint activity (Musacchio 2015) become transiently perturbed (Ninio 1991, Rosenberg, et al. 1998). For example, because the mitotic checkpoint is a global surveillance system that monitors the attachment of all chromosomes to the mitotic spindle, any stochastic defect in mitotic checkpoint activity renders every chromosome vulnerable to erroneous segregation at anaphase. A logical extension of this systemic vulnerability may be that during the

infrequent instances when the mitotic checkpoint does fail, multiple chromosomes can be mis-segregated during a single aberrant division to give rise to the complex karyotypes observed in our study of aneuploidization (Heasley, et al. 2020).

Decades of research have revealed how cellular processes such as those listed above work together to safeguard the integrity of the genome (Putnam and Kolodner 2017). Indeed, it is because of the efficacy of these pathways that cells maintain high-fidelity genome transmission for many generations without acquiring new mutations. However, our results indicate that on the rare occasions when such safeguards briefly falter, cells may experience an episode of SGI and acquire numerous mutations throughout the genome. Does this pattern represent punctuated equilibrium at the most fundamental level? Are the punctuated bursts we observed in our studies simply the result of stochastic failures in the very pathways that maintain prolonged genome stability (*i.e.*, stasis)? Additional work will be required to comprehensively investigate this concept, but if true, then perhaps punctuated equilibrium represents a mutational mode integral to eukaryotic genome evolution.

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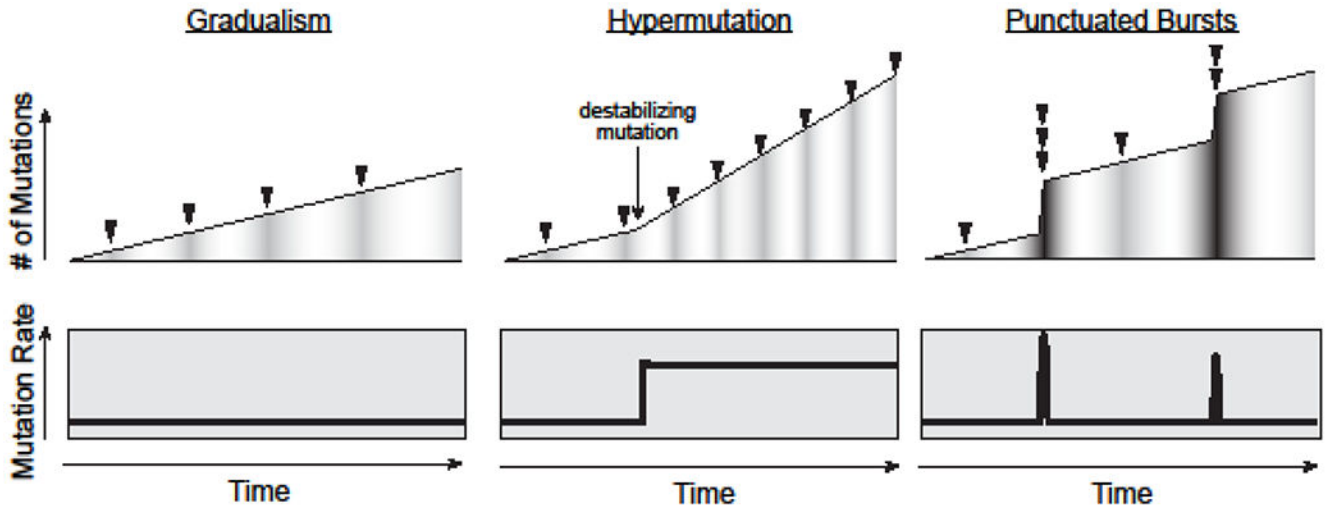

Fig. 1.

Darwinian gradualism (left) predicts that genomes acquire new mutations (arrowheads) sequentially and independently of one another (top) at linear and constant rates (bottom). The hypermutation model (center) predicts that following acquisition of a destabilizing "mutator" mutation, a genome may become chronically unstable and acquire new mutations independently at an elevated rate. The punctuated bursts model (right) predicts that genomes can experience discrete and transient episodes of systemic genomic instability (SGI) and acquire multiple new mutations rapidly but will resume stable genome propagation for extended periods.

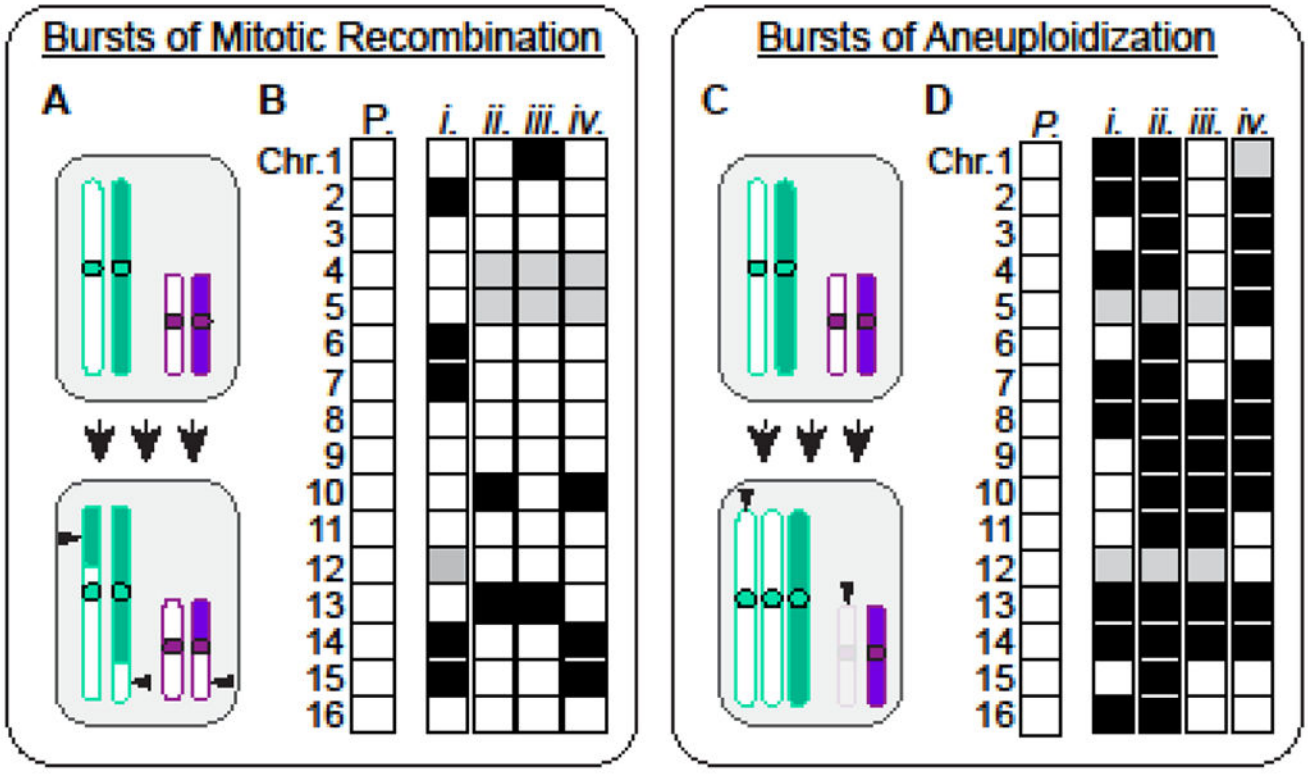

Fig. 2.

Yeast cells can acquire multiple mutations simultaneously. A) a cartoon illustrating a burst of mitotic recombination leading to multiple new LOH events. Shown are two pairs of homologous chromosomes (upper cell) and a derivative clone harboring numerous tracts of LOH (lower cell, arrowheads). B) Representative schematics of the parental diploid yeast genome and four selected clones that acquired multiple unselected tracts of LOH (Sampaio, et al. 2020). Each square represents both homologs of the denoted chromosome. White squares represent chromosomes that maintained the parental configuration. Grey squares represent the chromosome harboring the selected mutation. Black squares represent chromosomes that had concomitantly acquired an unselected tract of LOH. C) A cartoon illustrating a how a burst of aneuploidization can produce a clone harboring multiple aneuploidies. D) Representative schematics of the parental yeast genome and four selected clones that had acquired multiple unselected aneuploidies(Heasley, et al. 2020). White squares represent chromosomes that maintained the parental configuration (*i.e.*, one copy of each homolog). Grey squares represent the selected chromosome that was lost. Black squares represent chromosome pairs affected by unselected aneuploidy.