Figures and figure supplements

Quiescence unveils a novel mutational force in fission yeast

Serge Gangloff et al
Figure 1. Survival of $\text{ura}^-$ cells during two weeks of quiescence. Wild-Type, $\text{ura}4\text{D18}$ cells and a 50% mixture of both cultures were put into quiescence and their survival was followed for two weeks. $\text{ura}4\text{D18}$ has no advantage compared to wild-type strain or mixed populations and the proportion of uracil auxotrophs is maintained constant for the two weeks.

DOI: https://doi.org/10.7554/eLife.27469.002
Figure 2. Mutations accumulate as a function of time in quiescence. (A) slopes of FOA<sup>R</sup> accumulation as a function of time in quiescence from two independent experiments, determined by the least squares regression ($R^2 = 0.99$ for all mutations, $R^2 = 0.96$ for SNVs and $R^2 = 0.80$ for indels). (B) mutation spectrum established on 105 SNVs found in the <i>ura4</i><sup>+</sup> and <i>ura5</i><sup>+</sup> genes at day 1 and 146 at days 8 + 15. (C) distribution among indels and SNVs of the mutations that result in FOA<sup>R</sup> for <i>ura4</i><sup>+</sup> and <i>ura5</i><sup>+</sup> genes over time. (D) distribution of the various sizes of indels over time in quiescence. The numbers directly above the histograms indicate the number of events, while those on the top of the figure are the sum of all the events for each class. The numbers at the bottom indicate the size range of the indels.

DOI: https://doi.org/10.7554/eLife.27469.003
SNVs distribution. (A) Mutation spectrum established on 105 SNVs found in the *ura4*<sup>+</sup> and *ura5*<sup>+</sup> genes at day 1 and 146 at days 8 + 15 (from Fig. Figure 2B). (B) Same as in A, but normalized to the total number of relevant scorable base pairs. (C) Counts of all possible STOP and nonsense mutations for each substitution in *ura4* and *ura5*.

DOI: https://doi.org/10.7554/eLife.27469.004
Figure 2—figure supplement 2. Normalized percentage of each dinucleotide in the open reading frames of ura4+ and ura5+ after one day in quiescence. The percentage of every dinucleotide mutated at day one is compared to the frequency of occurrence of the same dinucleotide in the respective open reading frame.

DOI: https://doi.org/10.7554/eLife.27469.005
**Figure 3.** FOA<sup>R</sup> accumulation and corrected counts. (A) slope of FOA<sup>R</sup> (as determined in Figure 2A). (B) slopes of FOA<sup>R</sup> SNVs and indels accumulation. (C) corrected counts determined on all experimental points using least squares regression (D) corrected slopes for SNVs and indels formation in the *ura4<sup>+</sup>* and *ura5<sup>+</sup>* genes.

DOI: https://doi.org/10.7554/eLife.27469.008
Figure 4. Survival curves (red: linear; blue: log) of prototrophic cells in G0 as a function of time. The medium is being replaced every other week starting at day 15, while an aliquot is plated out to monitor the viability. The standard error of the experiments is indicated.

DOI: https://doi.org/10.7554/eLife.27469.013
Figure 5. Distribution of mutations among the 237 genomes sequenced. The six strains exhibiting a phenotype were excluded from the analysis. The fit between the observed and theoretical Poisson distribution indicates that Chronos acts homogeneously on genomes over time. The goodness of fit is excellent ($P_{\chi^2} = 0.56$).

DOI: https://doi.org/10.7554/eLife.27469.014
Figure 6. Corrected versus observed proportions of Indels. In blue, we report the percentage of indels expected from the FOA experiments, that is the ratio of the estimated slopes of accumulation of INDELS and SNVs (using method 1). In red, the observed fraction of INDELS after 3 months of quiescence. In green, the observed fraction of INDELs in the MA lines from (Farlow et al., 2015) P-values are computed using homogeneity chi2 tests.

DOI: https://doi.org/10.7554/eLife.27469.015
Figure 7. Mutation rate and spectrum in pcn1-K164R mutant strain.  
(A) Survival curves in G0 of 3 independent prototrophic pcn1-K164R clones as a function of time. The standard error is indicated. (B) Frequency with standard error and slope of FOA<sup>R</sup> accumulation in G0 of 3 independent pcn1-

---

**C**  
Non-redundant events  day 1  day 3  day 6  day 8  
SNVs  38  8  9  12  
insertions  0  3  6  3  
deletions  0  1  21  31  
complex  0  0  1  3  
total events  38  12  37  49  
%  day 1  day 3  day 6  day 8  
SNVs  100%  67%  24%  24%  
insertions  0%  25%  16%  6%  
deletions  0%  8%  57%  63%  
complex  0%  0%  3%  6%  
%  day 1  day 3  day 6  day 8  
SNVs  100%  67%  24%  24%  
indels  0%  33%  73%  69%  
complex  0%  0%  3%  6%  

---

**D**  
Percent of each class  
0  20  40  60  80  100  
day 1  day 3  day 6  day 8  
SNVs  100%  67%  24%  24%  
Indels  33%  73%  24%  24%  
Complex  69%  69%  69%  69%  

---

Figure 7 continued on next page.
K164R clones. The R square value is indicated. (C) Table and (D) graphical representation of the distribution among SNVs and indels of the ura4 and ura5 mutations accumulating over time in pcn1-K164R quiescent cells. The total number of the non-redundant events analyzed is indicated in the table.

DOI: https://doi.org/10.7554/eLife.27469.018
STOPs non-synonymous mutations with a phenotype synonymous

Figure 8. Schematic representation of a gene. In all three methods, the gene model is the same. We observe only a subset of all possible SNVs (the back areas) among all SNVs that lead to a phenotype (the black and grey areas). All synonymous SNVs do not exhibit a phenotype. The methods aim at estimating the unknown proportion $f$ of the non-synonymous SNVs that leads to a phenotype.

DOI: https://doi.org/10.7554/eLife.27469.019