Mutational load is ameliorated by increased transcriptional load-associated mutations, if these are biased towards duplications and deletions

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Abstract

Mutations can result from clashes between the transcription machinery and the replicative ones, on heavily transcribed genes, and can lead to chromosome rearrangements as well as point mutations. Transcription/replication conflicts lead to copy number variation in yeast's ribosomal gene, and their occurrence is under the control of a nutrient signalling pathway.

Here we study the effect of different mutational schemes on the evolution of a population of cells *in silico*, and show that transcriptional mutation biases towards duplications and deletions, such as those observed in yeast, are beneficial even though they increase the overall mutation rates. Moreover, we show that evolving a larger proportion of transcriptional duplications allows organisms to maintain high fitness in the presence of random, life-history independent high rates of deletions and deleterious mutations, as is the case for yeast rDNA.

1 Intro

Mutations do not occur uniformly over genomes [1], and their outcome often depends on their accessibility by the repair machinery [1–3]. Heavily transcribed genes, for instance, become often the stage for clashes between the transcription machinery and the replicative ones [4, 2].

Transcription/replication conflicts are known to lead to copy number variation in yeast's ribosomal gene count [2]. Moreover, it was recently discovered that the TOR pathway (a ubiquitous nutrient signalling pathway) controls ribosomal RNA gene duplications in yeast during caloric excess [5]. Interestingly, the signalling cascade leads to a larger rate of double strand breaks, which are then repaired by non-homologous recombination and results into a larger rate of duplications when the rDNA copy number is small. Furthermore, the increase (or decrease) of rDNA copy number does not confer an immediate selective advantage, hinting at second-order evolutionary effects.

Here we study the consequences of different mutational schemes on the *in silico* evolution of a population of cells, in order to understand the functional significance of the observed mutational regulation in yeast. We show that mutations induced by high transcriptional load are beneficial when they are biased towards duplications and deletions, even though they increase the overall mutation rates. Moreover, we show that evolving a larger proportion of transcriptional duplications allows organisms to

maintain high fitness in the presence of random, life-history independent high rates of deletions and deleterious mutations, as is the case for yeast rDNA.

2 Methods

We model a population of single-cell organisms with genome, proteome and a minimal regulome. There exists four types of genes, which code for enzymes (which convert resources into aminoacids), housekeeping proteins (which must be kept at a target homeostatic concentration), ribosomal RNA and ribosomal proteins (which translate transcription products by consuming aminoacids). The sum of the macromolecule constitute the cell volume. Competition favours cells with shorter inter-division time, i.e. the time cells take from birth to reaching a target volume, which is proportional to genome size. Distance from target homeostasis in housekeeping proteins gives a competitive disadvantage to cells.

Upon replication, different mutational regimes can be applied, the results of which are the concern of this study. In general, mutations can lead to gene duplications, deletions and inactivations. No back-mutations are possible from the inactive state, and inactive genes are still transcribed, but not translated.

3 Results

3.1

We simulated three model systems where cells are evolved under different mutational regimes. The first and simplest mutational regime assumes that mutations occur randomly and uniformly with a small, constant per-gene probability (we call these background mutations). We do not model any specific mechanism, but rather we focus on the outcome of mutations, which we assume be duplications, deletions and inactivations in equal proportion. The second model incorporates the observation that highly transcribed genes incur more frequently into mutations, because of conflicts between the transcription and the replication machinery [2]. We assume that heavily transcribed genes mutate frequently (while background mutations can still occur) and amplify background mutational effects. Essentially, if a clash between DNA polymerases and RNA polymerases occurs, double strand breaks may lead to gene inactivation [4] (we do not include larger-scale genome instabilities that threatens cell survival). The third model assumes that transcription/replication conflicts can be solved at special genomic regions that lie outside genes, such as replication fork barriers [6]. The effective outcome of mutations arising in these regions will mostly be duplications or deletions, because random insertions of nucleotide as a break-repair strategy will not destroy any open reading frame.

In Fig.1 we compare results for the systems evolved under these different mutational regimes, by collecting individuals along the ancestral lineage after reaching evolutionary steady state. Cells evolved with only a small rate of life-history independent mutations (μ_1 in Fig. 1) have, in general, slightly more inactive genes than active ones. In contrast, cells evolve to a much larger non-coding genome when transcriptional mutations amplify the effect of background mutations, while the count of coding genes decreases slightly (Fig. 1,μ_2). Moreover, this mutational regime leads to lower fitness, i.e. longer inter-division time, when transcriptional mutations amplify the effect of background mutations.

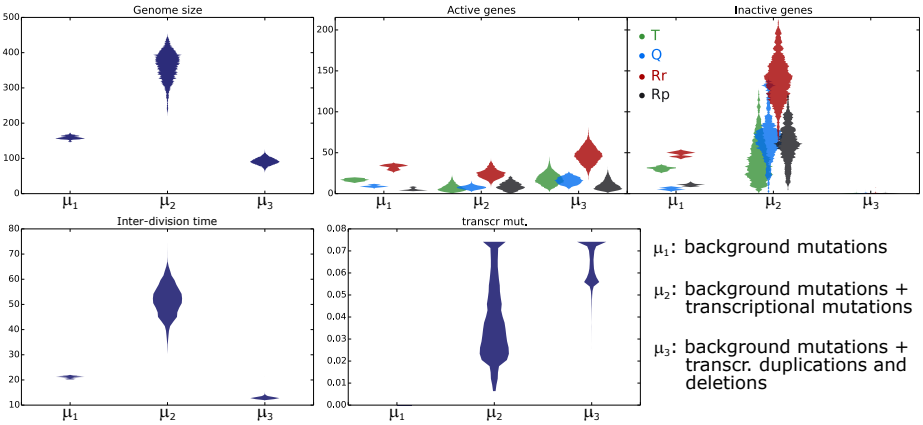

Figure 1: The evolution of high mutation rates can be selected if most mutations result in duplications or deletions.

Inactive genes are not purged from the genome because they contribute to decrease the transcriptional mutation rate, at the cost of a lower transcriptional load, which decreases the growth rate of cells, hence the longer inter-division time.

In sharp contrast, the effect of transcriptional mutations is beneficial when they increase the frequency of duplications and deletions, but not of inactivations (Fig. 1, μ_3). This is striking because the per-gene inactivation rate is the same as in the case of background mutations only. Cells replicate faster, and maintain a larger coding genome, while having virtually no inactive genes (inactive genes are purged quickly when they occur). Fast replication is achieved by keeping the transcriptional load to a high level, even though mutations occur more frequently. When the system was let evolve the relative proportions of transcriptional mutations, their evolutionary outcome was the same as those we imposed here (up to mutational fluctuations), while achieving similar fitness and genome composition (data not shown).

In conclusion, we make the case that larger mutation rates can be beneficial (and possibly selected for) when they result from the relocation of transcription/replication conflicts outside of the coding portion of genes, i.e. when the outcome of mutations is biased towards duplications and deletions.

3.2

It was recently discovered that the TOR pathway (a ubiquitous nutrient signalling pathway) controls ribosomal RNA gene duplications in yeast during caloric excess [5]. Interestingly, the signalling cascade leads to a larger rate of double strand breaks, which are then repaired by non-homologous recombination and results into a larger rate of duplications when the rDNA copy number is small. Furthermore, the increase (or decrease) of rDNA copy number does not confer an immediate selective advantage, hinting at second-order evolutionary effects.

Here we present some initial results by comparing two models. We first assume that only background mutations occur, they are frequent and result most often in deletions and inactivations (as occurs in rDNA [7, 8]). Secondly, we add transcriptional mutations and let the proportions of duplications, deletions and inactivations evolve.

In Fig. 2 we show that compact genomes with almost no inactive genes evolve in

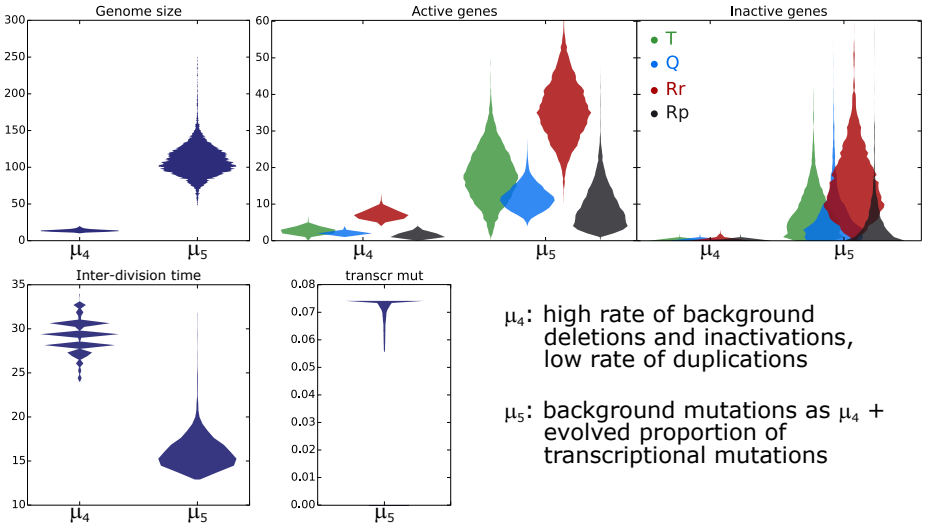

Figure 2: Evolution of mutational bias.

the presence of only background mutations, skewed towards deletions and inactivations (μ_4). The fixation of inactive genes is less successful when transcriptional mutations can evolve the relative proportions of mutational outcome (μ_5). Yet, in the latter case evolution reaches a larger final fitness (the inter-division time is about half of that without transcriptional mutations), despite a much larger genome, and, consequently, target volume.

The rate of transcription-induced duplications evolves to a slightly larger value than the rate of transcriptional deletions (not shown), most likely to balance the continuous loss of genes due to background mutations. We find that removing a large number of rDNA genes minimally reduces replication rates (not shown), in agreement with yeast rDNA genome dynamics [5]. Because removing genes can only increase the transcriptional load in our system, and because transcriptional mutations most often result in duplications, the gene copy number should increase and reach again it steady state copy number - like in yeast.

Altogether, we have shown that when life-history independent mutations are biased to deletions and inactivations, evolution skews the effect of mutations due to a large transcriptional load. In future work we will incorporate regulation of mutational outcomes in the model, as well as let resource availability change randomly, in order to study the evolution of regulated evolutionary dynamics as they happen in yeast.

3.3 Acknowledgement

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