University of Szeged
Faculty of Medicine
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Compensatory evolution as a driver of morphological novelties

Summary of the Ph.D. thesis

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Szeged
2022

List of publications and conference abstracts

Publication related to Ph.D. thesis:

Farkas, Z. †, Kovács, K. †, **Sarkadi, Z.** †, Kalapis, D., Fekete, G., Birtyik, F., Ayaydin, F., Molnár, C., Horváth, P., Pál, C. and Papp, B. (2022). Gene loss and compensatory evolution promotes the emergence of morphological novelties in budding yeast. Nat. Ecol. Evol. 1–11. https://doi.org/10.1038/s41559-022-01730-1.

† These authors contributed equally

Conference abstracts:

Z. Sarkadi*, Z. Farkas, K. Kovács, G. Fekete, C. Molnár, P. Horváth, D. Kalapis, Z. Bódi, C. Pál, B. Papp, Compensatory Mutations Drive Morphological Evolution, SMBE (Society for Molekular Biology and Evolution) in Manchester, UK, 23 July 2019.

Zsuzsa Sarkadi, Zoltán Farkas, Károly Kovács, Gergely Fekete, Dorottya Kalapis, Fanni Birtyik, Csaba Molnár, Péter Horváth, Ferhan Ayaydin, Csaba Pál, Balázs Papp, Deleterious mutations as drivers of morphological evolution, EMBL Conference: Molecular Mechanisms in Evolution and Ecology, 30 Sep - 2 Oct 2020.

Publications unrelated to Ph.D. thesis:

E. T. S. van, M. Forn, I. Forne, **Z. Sarkadi**, M. Capella, L. M. Caballero, S. Fischer-Burkart, C. Broenner, M. Simonetta, D. Toczyski, M. Halic, A. Imhof, and S. Braun, "Shelterin and subtelomeric DNA sequences control nucleosome maintenance and genome stability," EMBO REPORTS, vol. 20, no. 1, 2019.

R. R. Barrales, M. Forn, P. R. Georgescu, **Z. Sarkadi**, and S. Braun, "Control of heterochromatin localization and silencing by the nuclear membrane protein Lem2," GENES & DEVELOPMENT, vol. 30, no. 2, pp. 133–148, 2016.

Z. Sarkadi and M. Babits, "A megtermékenyülés és a spermiumalagút," TERMÉSZET VILÁGA, vol. 135, no. 6, pp. 259–262, 2004.

Introduction

Microbes show an immense diversity in cellular morphology. . However, the driving forces of morphological evolution in microbes are poorly studied. According to the traditional view, organisms adapt to the changing environment. However, recent studies suggest that beside environmental change, there might be other selective pressures that shape the phenotypes of organisms. Here we suggest an alternative scenario, where the environment is constant, and deleterious mutations induce selection pressure to compensate their harmful effects by specific mutations elsewhere in the genome, a phenomenon termed compensatory evolution. As a by-product, such genomic changes can lead to substantial divergence in morphological traits without direct selection acting on them.

To test our hypothesis we used a set of isogenic haploid *Saccharomyces cerevisiae* strains that had gone through laboratory compensatory evolution [1]. Each of these strains was initially slow-growing due to having one single gene deletion. These genes were involved in widespread biological processes. Here we measured the morphology of 86 slow-growing single-gene deletion strains (referred to as ancestor strains), and the corresponding 142 parallelly evolved lines (referred to as compensated strains). First, we focused on single cell morphology which reflects several cellular processes, such as cell size regulation, progression through the cell cycle and establishment of cell polarity [2]. Second, we measured multicellular morphologies, specifically, invasive growth, clump formation or biofilm formation. Studying the emergence of these traits are clinically important, because invasive growth and biofilm formation are virulence factors in pathogenic fungi.

Aims of the thesis

1. By measuring single-cell morphology using high-throughput microscopy and automated image analysis, we systematically tested whether compensatory evolution generated novel cellular morphologies (morphotypes).
2. Next, by cell cycle analysis of the ancestor and compensated strains using flow-cytometry we investigated whether the cell cycle phase alterations correlate with single-cell morphological traits, such as cell size and cell elongation.
3. By measuring three ecologically important multicellular traits, we tested whether compensatory evolution leads to the emergence of invasive growth, clump formation or increased biofilm formation.
4. Then, by measuring both single-cell morphology and multicellular morphology of yeast strains derived from nature, we tested whether the natural strains have comparable morphology to that of the laboratory-evolved strains.
5. Finally, by measuring genetic interactions at the level of morphology using two examples, we tested the existence of epistasis between gene deletion and compensatory mutation.

Methods I used in this work

1. **High-throughput DNA content analysis**. In order to exclude the compensated strains that became diploid during the evolutionary experiment, we measured the ploidy level of our strain set by flow cytometry by using SYTOX™ Green nucleic acid stain.
2. **Cell cycle analysis by flow cytometry.** To investigate potential changes in cell cycle phase distributions as a result of compensatory evolution, we analysed the DNA content of the compensated strains and their corresponding ancestors in using SYTOX™ Green nucleic acid stain. We assigned cells to specific cell cycle phases (G1, S and G2/M) and quantified the relative fraction of these cell cycle phases.
3. **Strain constructions for generating gene deletion and point mutation.** To investigate epistasis between *Δrpb9* and *Δwhi2* at the level of cell elongation, we deleted the *WHI2* gene both in the wild-type and the *Δrpb9* ancestral strains. Wild type *WHI2* allele was replaced by the *whi2::NatMX* cassette through homolog recombination.

In order to measure the genetic interaction between *Δbub3* and *SWE1*(*Y332S)* at the level of invasive growth, we used the the *delitto perfetto* method to introduce this point mutation into both the wild-type and the corresponding ancestor *Δbub3* background.

4. **Quantitative invasive growth assay.** We developed a high-throughput method to measure invasive growth of the compensated and ancestral strains. The non-invasive cells were washed off from the surface of the plates, and the amount of agar-embedded cells were estimated by image analysis.
5. **Quantitative settling assay to detect multicellular aggregates.** To systematically investigate the ability of the ancestor and compensated strains to form multicellular clumps or flocs, first we performed quantitative sedimentation assay. The fraction of the settled cells were estimated by using image analysis. Then the formation of multicellular aggregates were confirmed by microscopy. To distinguish between flocculation (calcium dependent aggregation) and mother-daughter separation defect (clumps), we performed deflocculation assay by using chelating agent (EDTA).
6. **Quantitative biofilm formation assay.** In order to investigate the biofilm formation ability of the ancestral and compensated lines, strains were cultured on low-density (0.3%) agar plates, and the area of the biofilms were estimated by image analysis.

Results

1. Rapid evolution of cellular morphology in the laboratory

By measuring the single-cell morphology of the compensated strains, we found that restoration of the wild-type morphology was relatively rare (15%), whereas in 46% of the cases, single-cell morphology of the compensated strains markedly differed from that of the wild-type and the corresponding ancestral strain. Changes in cell size and cell elongation were especially large. In addition, a distinct group of compensated strains showed very low bud neck position, indicating altered bud site selection in these cells. These results suggest that gene loss followed by compensatory evolution promotes divergence of morphological traits despite yielding wild-type like fitness.

2. Compensatory evolution alters cell cycle

By measuring the cell cycle phase distributions of the compensated strains, we found that 32% and 44% of the strains show a substantial alteration in G1 and G2 percentage, respectively. In addition, bud elongation was shown to be affected by the relative duration of G2 phase [3], and we found positive correlation between both bud and cell elongation and G2 percentage. These results suggest that compensatory evolution can alter cell cycle phase distributions and these alterations partly explain the observed morphological changes.

3. Rapid evolution of multicellular phenotypes

We studied three different forms of multicellular phenotypes: invasive growth, biofilm formation and clump formation. Our laboratory wild-type background wild-type fails to show invasive growth or cell aggregation phenotype, and forms 50% smaller biofilms compared to the positive control strain. Therefore, it is an ideal model to study the emergence of these phenotypes. Overall, 23.4% of the compensated strains showed an enhanced capacity to display at least one multicellular trait. Moreover, by measuring the invasive growth in a set of natural isolates, we found that extent of gain in invasive growth phenotype in the compensated strains reaches as high as ~50% of the range of invasiveness displayed by the natural isolates. These results indicate a prevalent role of compensatory evolution in generating multicellular phenotypes.

4. Comparable morphological diversity of compensated and natural strains

By focusing on three morphological traits; cell size, cell elongation and bud neck position, we compared the morphology of 29 natural *S. cerevisiae* strains with that of the compensated strains. We found, that the extent of morphological diversity in the laboratory-evolved strains was similar to that of natural isolates. This is quite surprising, because natural strains differ by ~10^2-10^5 mutations from each other, while the compensated strains typically differ by only ~6 mutations. We conclude that compensatory evolution in the laboratory generates morphological diversity in an exceptionally rapid manner (i.e. within ~400 generations).

5. Synergistic epistasis at the level of morphology

By measuring genetic interaction between gene deletion and compensatory mutation, we found synergistic interaction in both examples we examined. First, we found that elongated cell morphology of a compensated strain derives from the synergistic interaction between deletion of

RPB9 encoding an RNA polymerase subunit and the compensatory mutation occurred in *WHI2* encoding a negative regulator of TOR pathway. Second, by measuring invasive growth, we found that deletion of *BUB3* encoding a spindle assembly checkpoint protein also show synergistic interaction with the compensatory mutation in *SWE1* encoding a morphogenesis checkpoint protein.

Taken together, we found that deleterious gene losses promote emergence of new unicellular and multicellular morphologies, which arise as a by-product of compensatory evolution. Our work demonstrates that harmful mutations can play constructive role in morphological evolution by providing access to evolutionary routes that otherwise are not accessible.

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