Experimental approaches to study evolutionary cell biology using yeasts

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Abstract

The past century has witnessed tremendous advances in understanding how cells function. Nevertheless, how cellular processes have specifically evolved is still poorly understood. Many studies have highlighted surprising molecular diversity in how cells from various species execute the same processes, and advances in comparative genomics are likely to reveal much more molecular diversity than was believed possible until recently. Extant cells remain therefore the product of evolutionary history we vastly ignore. Evolutionary cell biology has emerged as a discipline aimed to address this knowledge gap by combining evolutionary, molecular, and cellular biology thinking. Recent studies have shown how even essential molecular processes, such as DNA replication, can undergo fast adaptive evolution under certain laboratory conditions. These approaches can open new lines of research where the evolution of cellular processes can be investigated experimentally. Yeasts naturally find themselves at the forefront of this research line. They not only allow us to observe fast evolutionary adaptation, but they also provide vast genomic, synthetic, and cellular biology tools previously developed by a large community. Here we propose that yeasts can serve as an *'evolutionary cell lab'* to test hypotheses, principles, and ideas in evolutionary cell biology. We discuss various experimental approaches available for this purpose, and how biology at large can benefit from them.

Introduction

Because of historical contingencies and technical limitations, evolutionary thinking has not been applied to cell biology as much as in other fields such as zoology and botany (Brodsky et al., 2012; Lynch et al., 2014; E. Richardson et al., 2015). The fossil record rarely provides a cell-level resolution (Donoghue, 2020), and most of what we currently know about cells derives from

the study of a handful of extant model organisms. These studies have been fundamental to identify common cellular processes as well as features that are unique to domains and kingdoms of the three of life. Most of the evolutionary cell biology has thus focused on how these general processes and features were first acquired during the early evolution of unicellular life and, perhaps, contributed to the general idea that housekeeping cellular functions evolved early and remained since then approximately unaltered (Lynch et al., 2014). However, a closer look at fundamental cellular processes in a few species is revealing a more nuanced view.

Although one could naively expect that a universal solution would exist for housekeeping essential processes and that such a solution was likely found early during the evolution of cellular life, different species seem to execute them in different ways. For instance, the cell cycle transition between the gap 1 (G1) and DNA synthesis (S) phase is tightly controlled in both budding yeast and mammals with a similar regulatory network. At the very core, Cyclin-Dependent Kinase (CDK) activity inactivates an inhibitor of cell cycle progression, leading to the transcription of the genes required to progress to the S-phase. Interestingly, some of the molecular players, such as transcription repressors (Whi5 and Rb), transcription factors (SBF/MBF and E2F) and CDK inhibitors (Sic1 and Kip-Cip) show no signs of sequence conservation between yeast and mammals (Cross et al., 2011). The G1/S transition commits cells to the replication of the genome, which requires two distinct processes: The *licensing* process equips chromosomal regions called 'origins' with the basic machinery required to start DNA replication. The *firing* process requires the activity of CDK, the loading of additional factors (among which are DNA polymerases), and promotes the beginning of DNA replication (M. O'Donnell et al., 2013). Preventing licensing outside of the appropriate time is essential to avoid the re-replication of chromosomal regions. Remarkably, this is achieved in different species through combinations of various strategies, including nuclear export, degradation, or post-translational modification of factors involved in licensing (Ikui et al., 2021; Kearsey & Cotterill, 2003). Throughout the cell cycle, cells protect their genomes from endogenous or exogenous sources of lesions through DNA damage and replication checkpoints. These are feedback control mechanisms that detect DNA damage and delay cell cycle progression to allow for DNA repair and tolerance (Branzei & Foiani, 2008; Pardo et al., 2017). These signaling cascades are composed of evolutionarily conserved kinases which, surprisingly, sometimes target different cell-cycle transitions and even respond to different signals in different organisms (Rhind & Russell, 2000).

These examples encompassing the regulation of DNA synthesis are just a few of the many cases where significant differences in the execution of essential processes between different

species have been reported. Collectively, these observations demonstrate the significant divergent evolution of the proteins involved in cell cycle regulation since the last eukaryotic common ancestor (Harashima et al., 2013). Occasionally, molecular complexes believed to be ubiquitous have even been found missing in certain species, challenging the very bases of our understanding of cell biology. For instance, chromosome segregation in eukaryotes requires the binding of centrosomes by the spindle microtubules. This process is mediated by specific centromeric histone variants (CENP-A), which are then recognized and bound by kinetochore proteins (such as the Ncd80 complex) connecting the chromosomes to the mitotic spindle (Drinnenberg et al., 2016; Santaguida & Musacchio, 2009). Remarkably, species of kinetoplastids have been found undergoing efficient chromosome segregation in the absence of such otherwise ubiquitous proteins (Akiyoshi & Gull, 2014). Another example is represented by the free-living protists metamonads, found to replicate their genomes without the otherwise essential origin recognition complex (ORC) and the licensing factor (Cdc6) (Salas-Leiva et al., 2021). Finally, the human pathogen *Giardia intestinalis* seems to undergo mitosis without an Anaphase Promoting Complex (APC), essential to trigger anaphase in all eukaryotes studied to date (Gourguechon et al., 2013). Altogether, these examples show how, even when the general architecture (i.e., the high-order organization) of an essential cellular process remains the same, its implementation (i.e., its molecular execution) can dramatically diverge during evolution.

To understand the magnitude of the divergence in housekeeping cellular processes, an intense sampling of the molecular variability existing in nature is paramount. Revealing a larger spectrum of molecular solutions, together with the genome sequencing of the organisms they are found in, will help decipher key questions about how cellular processes evolve (Goldstein & King, 2016). However, comparative studies won't inform on how such essential processes changed during evolution while avoiding the lethality often associated with mutations in several of their components. Furthermore, the simple observation of the outcome of an evolutionary process can't discriminate whether the evolved changes are the result of selective pressures, neutral processes, or historical contingencies (Blount et al., 2018). Finally, despite comparative genomics can estimate the time that occurred since the divergence of species, it can't further define when during this time a given change in a cellular process occurred, and how long, it took to be achieved.

Here we argue that while such a sampling endeavor is undergoing, experimental approaches in yeast can be used to answer some of the most important questions in evolutionary cell biology outlined above. In particular, the model organism *Saccharomyces cerevisiae* has several advantageous aspects that could serve this purpose. First, it is one of

the species in which cellular and molecular mechanisms are best dissected and understood. Being highly genetically amenable and tractable, *S. cerevisiae* facilitates forward and reverse genetic approaches, with a large set of molecular and cellular tools and reporters that have been developed by a large and diverse community. Its well-annotated and relatively small genome facilitates genomic analyses and allows for inexpensive sequencing of large sets of strains (Duina et al., 2014). The existence of large panels of well-characterized and culturable natural isolates (Peter et al., 2018), as well as closely related species (Scannell et al., 2011), provides a detailed window over the species' evolutionary history. Importantly, the molecular diversity in cellular processes is not limited to the one that has been sampled in nature so far, but can be further extended in the laboratory by exposing cells to artificial selection (Figure 1, red arrow). Yeasts' fast generation time facilitates experimental evolution approaches under precise laboratory conditions and specific selective pressures, which allows the identification of possible causes of variation underlying key cellular mechanisms. Some of these experiments have recently challenged the view that basic cell biology evolves over geological timescales by showing how alternative solutions to some of the most conserved cellular processes can be found by cells over only hundreds of generations (Fumasoni & Murray, 2020; LaBar et al., 2020).

Because of these advantages, we propose that yeasts could serve as an '*evolutionary cell lab*' to test hypotheses about the evolution of molecular processes and to investigate the 'rules of the game' which dictate the evolution of cell biology at large. To this end, we review some of the approaches that have been recently employed and highlight how they can provide answers about the evolution of cellular mechanisms. This piece focuses on work providing insights on the evolution of cellular processes and their execution by molecular machines, rather than on genome and sequence evolution (which have been reviewed elsewhere (Scannell et al., 2007; Seoighe & Wolfe, 1999; Todd et al., 2017)). The examples provided here are related to cell division and the maintenance of genetic material, but the approaches described are applicable, in principle, to the study of any cellular process.

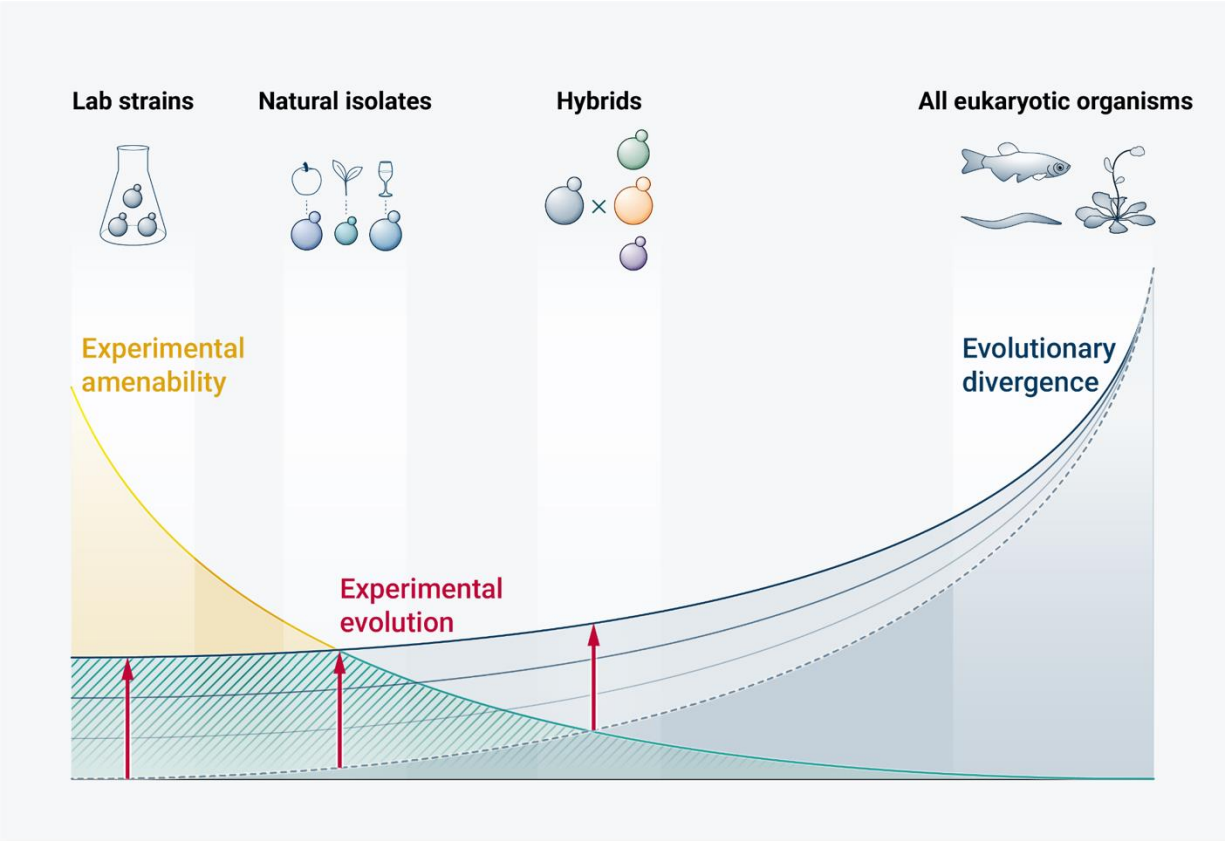

Figure 1. The 'sweet spot' for experimental evolutionary cell biology. Experimental amenability (yellow area) decreases proportionally to the biological distance of the specimens with laboratory model organisms (*S. cerevisiae* in this representation). Evolutionary divergence (blue area), on the contrary, is the highest among a large group of eukaryotic species, progressively decreasing in organisms sharing a recent evolutionary history, approaching zero in the small set of commonly used laboratory yeast strains. Experimental evolution approaches (red arrows) can be used to generate evolutionary divergence in the laboratory, thus expanding the green area (overlap between yellow and blue) which represents the optimal region to investigate evolutionary cell biology experimentally. (Distances are not at scale to emphasize the green area).

Yeast Hybrids & Natural Isolates

Genetic diversity between species, or among individuals from the same species from different ecological niches, can be highly informative on the evolution of key cellular processes. When genetically diverse individuals are crossed, they can produce hybrids that are sterile and/or present significant growth defects, depending on the degree of genetic incompatibility of their parents (Figure 2A). These incompatibilities can arise as a consequence of large-scale genome reorganization events such as aneuploidy, chromosomal rearrangements, or as a

result of the parents' divergence in key cellular processes (Bozdag & Ono, 2022). The ease in mating and phenotypic analysis of yeast hybrids greatly facilitates dissecting the origins of molecular incompatibilities. In particular, incompatibilities due to divergent cellular processes can shed light on their evolution in the parental species (Chou et al., 2010).

Zill and colleagues crossed *S. cerevisiae* and *S. bayanus* to gain an understanding of the evolution of transcriptional silencing, a process essential for heterochromatin formation and mating locus silencing in yeast. At the heart of this process acts a complex of Silent Information Regulator (SIR) proteins, Sir2, Sir3, and Sir4. Interestingly, unlike Sir2/3, Sir4 protein interactions differ between these closely related species, as evidenced by the inability of *S. cerevisiae* Sir4 (Sc-Sir4) to complement *sir4* mutants in *S. bayanus*. Functional assays in interspecies' hybrids gave further insight into the cis-acting differences between the two species' silencers, showing that Sc-Sir4 cannot stably associate with *S. bayanus* intermediate proteins nor promote the silencing of its mating locus. This asymmetric non-complementation between *S. bayanus* and *S. cerevisiae* suggests a functional divergence of Sir4 and other silencing proteins over recent evolutionary history, which may have shaped the co-evolution of the silencing proteins to always ensure a proper sexual cycle and heterochromatin formation in both species (Zill et al., 2012, 2010).

Natural populations of the same species isolated from different ecological niches offer another useful source of genetic diversity (Figure 2B). Peter and colleagues recently sequenced 1011 genomes from *S. cerevisiae* strains isolated from a selection of global natural and domesticated niches (Peter et al., 2018). This dataset has been fundamental for the understanding of genome evolution across natural isolates, but it has not been yet fully exploited as a powerful tool for the functional characterization of cellular differences among these strains. Several 'wild' alleles carrying differences in housekeeping processes have been reported by Parts and colleagues. One example is represented by *NSE1-UW*, which encodes for a component of Smc5/6, a highly conserved and essential complex with a key role in genome maintenance (Lehmann et al., 1995; Sergeant et al., 2005). The wild *NSE1* allele was identified in a panel of natural isolates due to its ability to suppress mutations in other Smc5/6 components, namely in Nse3 and Nse4. Further investigation showed that *NSE1-UW* can suppress the partial loss of function of these SMC components by increasing the recruitment of these complexes to the DNA template (Parts et al., 2021). These results illustrate how short-term evolutionary history can produce essential complexes with altered protein stoichiometry.

Similarly, Bui and colleagues took advantage of the recently sequenced library of *S. cerevisiae* wild isolates to detect variants of the Mlh1-Pms1 heterodimer, involved in mismatch repair. Previously, the combination of two alleles in *MLH1* and *PMS1* genes was reported to cause an increase in mutation rates, which was detrimental in rich media (Heck et al., 2006) but slightly advantageous under stressful environments (Bui et al., 2015). Interestingly, 18 of the isolates screened carried the two alleles in heterozygosity and only a clinical isolate was found to carry them in homozygosity (Bui et al., 2017). This suggests how the mismatch repair pathway may have evolved alleles of the Mlh1 and Pms1 subunits of the heterodimer capable of generating various levels of mutation rates. In particular, the authors proposed how the occasional combination of the two alleles can be achieved to generate hyper-mutator phenotypes advantageous in stressful environments, such as the one of a hospital. This transient mutator phenotype can then be later separated from the beneficial mutations it induces, upon allele segregation after mating or by accumulating suppressor mutations.

Altogether these works provide examples of how yeast interspecies hybrids and natural isolates can be exploited to dissect the natural phenotypic variation in cellular processes, its genetic basis, and its putative dependence on ecological niches (Figure 2A-B).

Gene swaps

The efficiency of homologous recombination in yeast allows for the fast replacement of native genes with the ones of other species, an approach defined as ‘gene swap’. This tool can be readily used to demonstrate the degree of conservation of a protein function across several evolutionary timescales (Figure 2C).

A striking example of the power of this approach is represented by the complementation of the lack of *CDC2* in *Schizosaccharomyces pombe* (encoding for Cdk1), with its human homolog, demonstrating the conservation of the basic engine driving the cell cycle between yeast and humans (Lee & Nurse, 1987). In principle, a heterologous protein can retain any degree of function conservation, ranging from full complementation to unviability. For instance, Zamir and colleagues studied the conservation across fungal species of the Proliferating Cell Nuclear Antigen (PCNA), an essential protein that plays a crucial role in DNA replication and repair through interaction with multiple partners in a well-characterized protein-protein interaction (PPI) network (Choe & Moldovan, 2017). The authors focused on the PCNA domain IDCL, already described to mediate several PCNA-partner interactions (Fridman et al., 2010). To understand the degree of functional conservation of this domain, chimeric PCNA (cPCNA)

proteins were engineered by swapping the IDCL domain with the one of other yeast species. The chimeric PCNA were then heterologously expressed in *S. cerevisiae* under its native promoter. Cells expressing cPCNA with IDCLs from species closely related to *S. cerevisiae* showed no growth defects, while those expressing cPCNA from *A. nidulans* and *N. crassa* were unviable. Interestingly, strains with cPCNA from *Y. lipolytica* or *S. pombe*, which are evolutionarily more distant to *S. cerevisiae* than *N. crassa* or *A. nidulans*, were viable but showed defects in DNA replication and repair. Full-length PCNAs from *Y. lipolytica* or *S. pombe* also manifested increased sensitivity to DNA-damaging agents, while retaining equal or higher affinity than scPCNA to the most important interaction partners (Zamir et al., 2012). This work emphasizes how the functionality of the PCNA PPI network does not correlate with evolutionary distance alone, and how its evolution depends on the fine-tuning of the affinities between protein binding domains and their binding partners.

The centrosome is another conserved eukaryotic cellular structure, and usually comprises two main elements that interact with each other: centrioles and an electron-dense proteinaceous pericentriolar material (PCM). The fission yeast *S. pombe* lacks centrioles but builds a functional centrosome solely with a PCM-like structure, known as the spindle pole body (SPB). The maintenance of the PCM in fission yeast provides a powerful tool to understand the evolution and plasticity of this key cellular structure (Cavanaugh & Jaspersen, 2017). Ito and colleagues engineered *S. pombe* to heterologously express *Drosophila melanogaster* centriole components, demonstrating that the yeasts' SPB retained the ability to recruit them, maintaining a residual centriole-like function. The authors further validated some of these interactions in the fruit fly, showing that the location of the centrioles is not only determined by the positive feedback loop in its components, as previously thought, but through a conserved regulatory network between centriole components and the PCM (Ito et al., 2019).

Gene swaps can also be employed to dissect the functional divergence of paralogue proteins within the same species. Scc1 and Rec8 are two paralogs from the kleisins family and are involved in sister chromatid cohesion during mitosis and meiosis, respectively. To explore their functional divergence, Hsieh and colleagues induced *REC8* expression in mitosis by swapping its promoter with the one of *SCC1 (pSCC1-REC8)*. The mitotic expression of the meiotic kleisin produced a clear fitness reduction and perturbed cell division by impairing sister chromatid cohesion and DNA replication, highlighting how Rec8 had partially lost some of the important functions of Scc1. The authors mitotically evolved strains carrying *pSCC1-REC8* to test whether these functions could be readily recovered by Rec8. Instead, it was found that cells acquired adaptive mutations in transcriptional mediators, cohesin-related genes, and G1/S

regulators (Hsieh et al., 2020). This study uncovered a novel function of kleisin proteins in regulating the speed of S phase and suggested a possible reason for the divergence of Scc1 and Rec8 to support the mitotic and meiotic cell cycle.

These experiments highlight how gene swaps in yeast can be used to test the extent of conservation of ancient cellular functions, proteins' residual interaction networks, and their functional relevance for the cellular process they take part in. The steep progress in sequenced genomes is providing increasing high-quality genomes of species from different kingdoms (Lewin et al., 2022). Gene swap approaches could take advantage of these large genomic resources and provide a tool to gain further functional insights about proteins involved in housekeeping processes across the tree of life.

Bypassing gene essentiality

Housekeeping processes are enriched in essential genes, which are traditionally defined as required for cellular viability and reproductive success. However, assigning this label to any gene is less straightforward than one would think. Genes can be essential in one species but not in others (Rosconi et al., 2022; Ryan et al., 2013), or only in specific environmental conditions (Baba et al., 2006). Furthermore, studies in yeast have shown how a subset of mutants in essential genes can retain viability via fast adaptive evolutionary processes (Liu et al., 2015; Rancati et al., 2008; Van Leeuwen et al., 2016, Van Leeuwen et al., 2020). Liu and colleagues found that the deletion of 9% of the genes reported as being essential in *S. cerevisiae* generated some viable cells, most of which recovered fitness by acquiring aneuploidies during short evolutionary experiments (Liu et al., 2015). In particular, mutants of the nucleoporin complex, which facilitate the transport of molecules through the nuclear pore, recurrently accumulated extra copies of chromosome VIII. This aneuploidy was shown to be driven by the necessity of the mutants to carry at least one extra copy of the gene encoding Brl1, which compensates for the lack of functional nucleoporin by altering the nuclear membrane fluidity. This study showed how gene essentiality is a quantitative, rather than a qualitative property, and that changes in protein abundance can quickly provide an adaptive route to a number of mutants in essential genes. Alleles bypassing the essentiality of 124 genes were also found in a suppressor screen that took advantage of the yeast synthetic genetic array (van Leeuwen et al., 2020), and gene variants found in natural isolates were later found to suppress the lethality of hundreds of essential genes (Parts et al., 2021). Studying in detail how the essentiality of specific genes can be bypassed can shed light on cellular processes that are

more likely to be altered to rescue viability and thus the mechanisms by which lethality can be avoided. A complete view of how essentiality can be bypassed will eventually offer potential mechanistic explanations for the remarkable cases of loss of essential complexes which have been or will be reported in the coming future.

Evolutionary repair

'Evolutionary repair' defines a class of experiments in which experimental evolution is used to adapt cells to a targeted cellular stress. When a cellular process is impaired by a genetic perturbation, the organismal fitness decreases to an extent that is proportional to the number and severity of the cellular defects it produces. Experimental evolution is then performed by the continuous growth of parallel populations of mutants. Mutations occurring spontaneously, which increase cellular fitness and survive genetic drift, are expected to spread within the populations. The successive accumulation of such mutations leads to progressive increases in fitness, which can eventually approach (Fumasoni & Murray, 2020), or even surpass (Helsen et al., 2020) the one of the unperturbed wild type after many generations (Figure 2D, for a detailed review on the design and execution of evolutionary repair experiments we refer to (LaBar et al., 2020)). This experimental methodology allows an understanding of how cells 'repair' the original perturbed process by generating alternative solutions. These, in principle, can be obtained in two distinct ways: The first class of solutions is to evolve to recover the function(s) impaired by the perturbation, and the second class consists of evolving to a state where the impaired function(s) is no longer needed.

An example of the first class solution was recently reported in (Pavani et al., 2021), where strains with three amino-acid substitutions in the essential microtubules' subunit Tub2, mimicking the effect of antimitotic drugs, were evolved. While evolved strains carried several adaptive mutations, the authors found that the most frequent and efficient strategies were amino-acid substitutions (but not reversion) in the *TUB2* gene, which corrected the initial defect and re-stabilized microtubules. However, examples of the second class of evolutionary repair are more abundant. One is represented by (Laan et al., 2015) where cells were adapted to the absence of Bem1, a protein involved in cell polarization. Bem1 brings Cdc42 in proximity with its activator Cdc24, at a site of the membrane, which, upon Cdc42 activation, is chosen as the next cytokinesis site. ***bem1Δ*** strains have severe problems in choosing a polar axis and thus divide very slowly, causing a marked decrease in fitness. The evolutionary repair led to frequent loss of function mutations in *BEM2*, *BEM3,* and *NRP1*, which belong to the same functional

module and whose products inhibit Cdc42 activity. This work offers an example of how a cellular process can be evolutionarily rewired by altering the relationships between activators and inhibitors within the same module. However, other studies show how the adaptive mutations acquired during evolutionary repair are not always limited to the same cellular module perturbed. Fumasoni and Murray followed the evolutionary adaptation to constitutive DNA replication stress, imposed by the absence of Ctf4 (Fumasoni & Murray, 2020), a structural hub coordinating the activities of the enzymes at the DNA replication machinery (Fumasoni et al., 2015; Simon et al., 2014; Villa et al., 2016). Evolved strains recovered from the severe fitness defect imposed by the absence of Ctf4 by frequently acquiring mutations in three distinct modules: sister chromatid cohesion (*scc2*), DNA replication (*sld5* and *ixr1*), and cell cycle progression (*rad9*). These evolutionary trajectories were found to be very reproducible across several replicates, even when the genotype of the initially perturbed strains varied in ploidy and in recombination proficiency (Fumasoni & Murray, 2021). These results show how an initial genetic perturbation can cause a series of compensatory adaptive mutations which penetrate cellular modules that are functionally connected to the one perturbed, resulting in a re-wiring of a greater system, in this case, genome maintenance at large.

Other work used an evolutionary repair approach to study other important and conserved cellular processes. Frumkin and colleagues selected strains with a gene carrying an inefficient intron for higher gene expression. Interestingly adaptive mutations increased splicing efficiency by mutations in *cis* (within the intron sequence), generating mRNA structures that were easier to splice, and in *trans* (in the rest of the genome), increasing the efficiency of the splicing machinery by affecting the binding of two subunits to the mRNA (Frumkin et al., 2019). A number of other evolutionary repair experiments were performed challenging cytokinesis (Rancati et al., 2008), mitochondrial function (Amine et al., 2021), and chromosome segregation (Ravichandran et al., 2018). Furthermore, large-scale experiments evolving mutants in many other cellular modules have been performed (Helsen et al., 2020; Rojas Echenique et al., 2019; Szamecz et al., 2014).

Altogether these experiments show how, at least under laboratory conditions, even some of the most essential and conserved cellular processes can quickly change upon exposure to a specific stress. By extending this logic, it is tempting to speculate that virtually every aspect of cell biology could be subjected to substantial changes over short evolutionary timescales. Importantly, these experiments have revealed that adaptive strategies have a certain degree of reproducibility. This means that evolutionary repair experiments can be used to predict which and how many alternative solutions to a given process exist, and their relative probability of

appearance and persistence in a population. If properly designed, evolutionary repair experiments can therefore be used to generate hypotheses regarding the origin of specific cases of molecular variability found in nature. Furthermore, the predicting power of these experiments can serve as a powerful tool to foresee how cell biology will adapt to future challenges, including many relevant to human health and climate change (Fumasoni, 2020; Lässig et al., 2017; Wortel et al., 2021).

Conclusions and future perspectives

The studies summarized above offer an example of how decades of findings and tools developed in yeasts can be capitalized for the experimental study of evolutionary cell biology. We argue that these sparse examples represent only a small fraction of the research possibilities in this field, and that a larger set of tools developed by many laboratories working in cell biology are yet to be exploited in combination with the approaches outlined here. Furthermore, continuous developments within the yeast community are likely to further expand the range of opportunities to experimentally investigate the evolution of cellular processes. While traditionally considered suboptimal for the imaging of intracellular features, super-resolution techniques were recently developed (Chen et al., 2021; Hinterndorfer et al., 2022; Korovesi et al., 2022), and used (Dey et al., 2020) in yeast to study cellular processes at higher resolution, and new tools have been provided for the imaging of several subcellular structures (Akhuli et al., 2022; Zhu et al., 2019). A large number of telomere-to-telomere assemblies recently revealed the landscape of structural variants of *S. cerevisiae* natural isolates (S. O'Donnell et al., 2022). Finally, synthetic biology approaches such as The Synthetic Yeast Genome Project (S. M. Richardson et al., 2017) continue to provide novel tools to generate synthetic cellular conditions. We propose that the research lines outlined here hold the potential to reveal the laws dictating the evolution of molecular processes, and jointly with extensive comparative studies, will shed light on the very basis of cellular life.

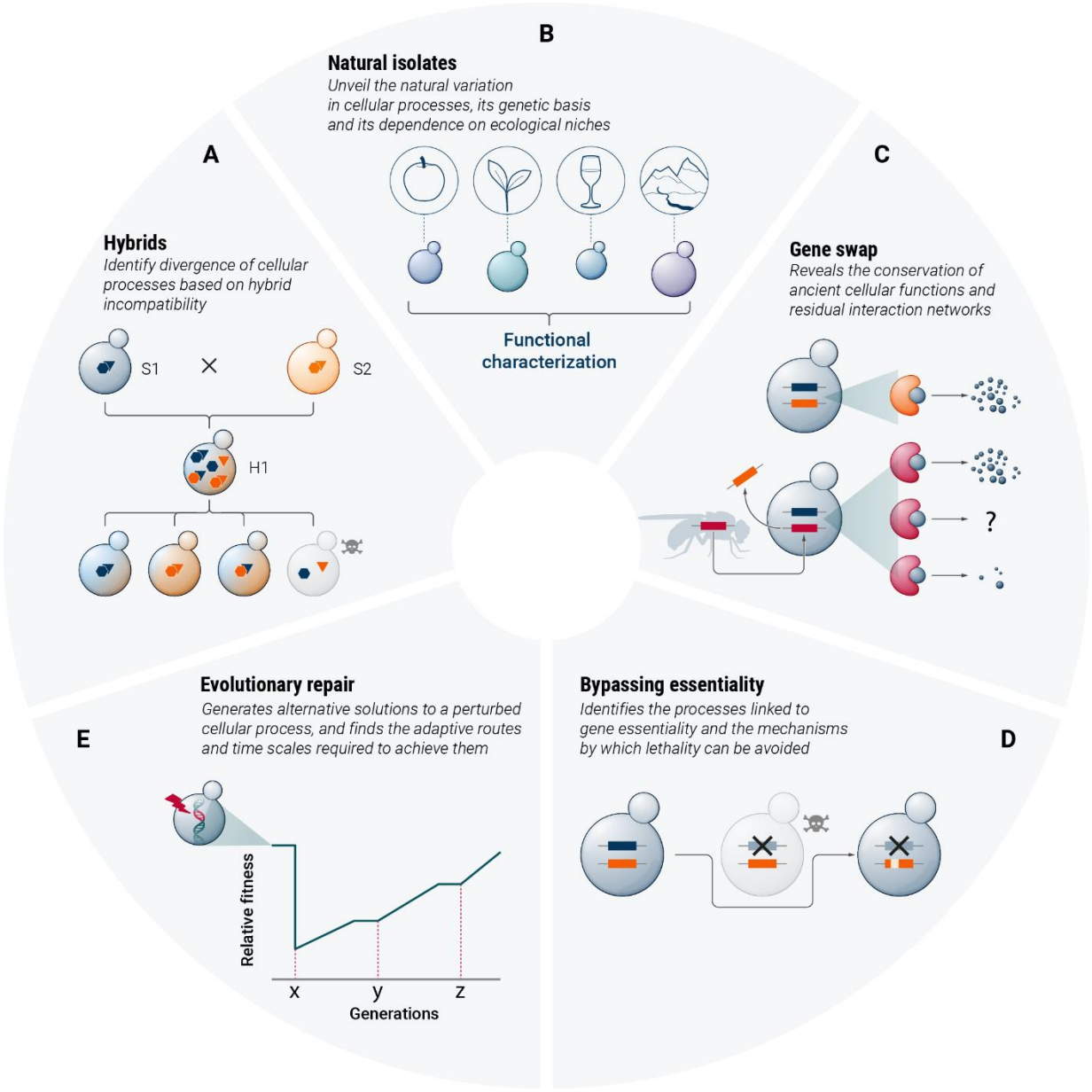

Figure 2. Experimental tools for studying the evolution of cell biology using yeasts. (A) Different yeast species (S1 and S2), which present genetic variation for a given cellular mechanism are crossed to generate a diploid hybrid (H1). Upon sporulation, it is possible to identify genetic incompatibilities, demonstrating evolutionary divergence. The orange triangle, originally encoded in S2 cannot bind to the blue hexagon encoded by S1, while the blue triangle from S1 retains the ability to bind its partner encoded by S2. (B) Functional characterization of strains isolated from different ecological niches, which carry alleles responsible for variability in cellular processes. (C) Gene from *Drosophila melanogaster* (red) replaces the native gene of *S. cerevisiae* (orange). Functional studies in yeast are then conducted to understand the degree of functional conservation of the gene product. (D) Deletion of an essential gene

(blue) leads to cell death, which can be bypassed if modifications occur in other genome loci (white band in orange locus). (E) Genetic perturbation causes a fitness reduction. Experimental evolution allows cells to recover this fitness loss through a sequence of compensatory changes in their genome (represented as Y, X, and Z). Sentences in italics emphasize some of the evolutionary cell biology aspects that can be learned with each approach.

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