Insights into molecular evolution from yeast genomics

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Abstract

Enabled by comparative genomics, yeasts have increasingly developed into a powerful model system for molecular evolution. Here we survey several areas where yeast studies have made important contributions including regulatory evolution, gene duplication and divergence, evolution of gene order, and evolution of complexity. In each area we highlight key studies and findings based on techniques ranging from statistical analysis of large data sets to direct laboratory measurements of fitness. Future work will combine traditional evolutionary genetics analysis with experimental evolution with tools from systems biology to yield mechanistic insight into complex phenotypes.

Keywords

molecular evolution, comparative genomics, experimental evolution, complex phenotypes, population genomics, gene duplication, gene order, regulatory evolution, functional synthesis, functional genomics

Introduction

The beginning of "comparative genomics" was a turning point for molecular evolution. Two types of genome sequences were most often compared: those of "closely" related species whose entire genomes could be aligned at the level of the nucleic acids (116) and genomes of more "distantly" related species that showed interesting variation in lifestyle and physiology, but were close enough that most genes had clear orthologues (3). Because budding yeast was the first eukaryote to have its genome completely sequenced (38), it was naturally at the forefront of the comparative genomics work [(18), (19), (53), (27), reviewed in (33), with some genomes re-sequenced recently (101)]. The hemiascomycetous yeast species whose genomes are now available span a similar evolutionary distance as the chordates (27), making these genomes a model system for animal evolution.

Comparative genomics vastly expanded the scope of molecular evolution. The availability of evolutionary measurements for thousands of genes could be used to evaluate evolutionary hypotheses in general using statistical analysis (120), as opposed to analyzing single genes anecdotally as had often been done before. However, comparative genomics also allowed entirely new types of molecular evolution analysis: the order of genes, the large stretches of non-coding DNA, and the organization of pathways and regulatory networks. Comparative genomics also enabled functional genomics in multiple species, leading to a further expansion of the questions that could be tackled: evolution of genome-wide expression patterns, protein interactions and post-translational modifications. Once available, the comparative sequence and functional data could be applied in several areas of interest in molecular evolution. For example, one of the fortuitous discoveries made soon after the completion of the yeast genome was the identification of a whole genome duplication event (121). The comparative data for this set of gene duplicates ("ohnologs") continues to allow unprecedented large-scale studies of gene duplication and divergence, facilitating studies of classical topics in molecular evolution (54).

More recently, both comparative and functional genomics techniques have been applied to genomes of yeast strains (the rough equivalent of individuals in multi-cellular populations), heralding the era of "population genomics" (68). Once again, unprecedented genome-scale

measurements of nucleotide diversity, allele frequencies and functional differences between individuals opened new areas of research to evolutionary geneticists.

Because of the power and relative ease of experiments in yeast, it is increasingly a model organism of choice for mechanistic evolutionary studies, in which the evolutionary history of molecular function is reconstructed in the lab, and fitness of populations or alleles is measured directly (22). Studies including so-called "experimental evolution", where the evolutionary process can be directly observed and manipulated in the lab, are leading to a new expansion in the scope of questions that can be addressed. Although still limited by the lack of spectacular evolutionary novelties (such as fur, flight, camera eyes, social lifestyle, language, etc.), yeast has recently been used as an experimental model for evolution of complex phenotypes, such as multi-cellularity, memory and co-operation (reviewed in 16). Given the resources and techniques available, we believe the future scope of this work is limited only by the creativity of the researchers.

Here we survey these areas where yeast has had an impact in molecular evolution, and where we think the most exciting future questions for yeast molecular evolutionists lie. For the most part, we will point the reader to recent reviews in each area of molecular evolution, and highlight specifically only some exciting recent articles.

Big yeast data for evolutionary biology

Several important questions in molecular evolution have been evaluated statistically using yeast data. The systematic data about nearly every aspect of yeast gene function have made yeast a powerful tool for all basic statistical studies of the relationship between evolutionary properties and molecular function.

A longstanding question in molecular evolution is why rates of protein evolution vary over orders of magnitude (see 17 for review). The large sets of measurements of rates from yeast proteins available because of the "closely" related genomes, combined with the functional genomics data allowed identification of features that were correlated with rates of protein evolution, and therefore potential causes of the rate variation. For example, using yeast data, gene expression levels were found to be strongly correlated with evolutionary rate (83). This eventually led to the model that evolutionary rate is in part determined by the propensity of proteins to misfold and that this effect is strongest for highly expressed proteins (26). Follow-up experimental studies have quantified this effect in yeast (35). The rate of protein evolution was also found to be influenced by the number of physical interactions and binding interfaces available (29, 55), as well as mRNA folding strength (85). More recently, it has been suggested that selection against spurious protein interactions might also contribute to the correlation between expression levels and evolutionary rate (124). A related question is whether the genes that are most important to survival, which can also be measured systematically in yeast (36), show slower rates of evolution. Surprisingly, although it is statistically significant, the data has shown only a weak correlation between "essentiality" and rate of evolution (115).

Other interesting recent examples include use of genetic interaction data to study the impact of epistasis on gene expression evolution (76), use of ribosomal profiling data to directly test long-standing models for selection on codon-usage (93), genome-wide recombination rate estimates to examine the effects of recombination on selection (21) and mutation (61), and statistical analysis of eQTLs to infer selection on gene expression levels (14, 31).

Yeast and regulatory evolution

Comparisons of the gene numbers between model and human genomes supported the idea that increases in complexity of non-coding DNA and gene regulation might be more important to evolutionary increases in organismal complexity than gene number (66). Despite the excitement about regulatory evolution, what was known about the evolution of non-coding DNA prior to comparative genomics was largely anecdotal (reviewed in 123). Analysis of the "closely" related yeast genomes showed that functional non-coding DNA was conserved, but that over longer evolutionary distances there was little conservation of regulatory sequences at the DNA level. Gene expression and other genomic data for multiple species have supported the idea that gene regulation has changed considerably among yeast species (reviewed in 117, 118).

Facilitated by comparative genomics, the first evolutionary studies of specific gene regulatory networks soon appeared, with yeast serving as the model for several of the most interesting ones (reviewed in 67). A number of studies have attempted to quantify the relative abundance of cis versus trans evolution in yeast (111, 112, 14, 30, 31, 74). Perhaps most surprisingly, several examples of trans- regulatory evolution were identified, where transcription factor specificity co-evolved with a large number of DNA binding sites [Rpn4: (34), AP-1: (59), Matα1: (6)]. These types of changes are highly pleiotropic and therefore (before they were discovered) were expected to be very rare (90, 123). Recently, several mechanisms of regulatory evolution have been characterized in the cell-type specification system (4, 9) and the ribosomal transcriptional control network (65, 72). Along with the galactose utilization (*GAL)* network, which has revealed several surprising and important patterns of evolution (47, 75, 45, 46, 52, 84), these yeast regulatory networks are now among the best understood systems for regulatory network evolution.

Recently, proteomics experiments have begun to characterize evolution of signaling networks, also referred to as regulatory evolution at the post-translational level (reviewed in 7). Like transcriptional regulatory sequences, post-translational regulatory sites are apparently largely conserved between "closely" related species of yeast (48, 79, 80). At further evolutionary distances, some modifications and interactions show high levels of divergence (8, 48, 109), while other protein-protein interactions evolve much more slowly (91). Further research will be needed to determine the major patterns of protein regulatory evolution, but it is clear that regulatory evolution at levels other than transcription is an emerging area (78), with yeast a leading model system.

Gene duplication and divergence after the whole genome duplication

The comparative genomics resources based on yeast gene order conservation, and the existence of the whole genome duplication, allowed reconstruction the evolutionary history of a large number of duplicate gene pairs and study of their patterns of divergence from the single copy ancestors (99, 100). In addition to a burst of rapid molecular evolution following gene duplication (98), increased speciation was also apparently associated with the whole genome duplication (100).

The set of gene duplicates also facilitated studies that contrast the genes that were retained in two copies with those whose duplicate copies (the vast majority) were lost after the duplication. The earliest analysis revealed intriguing functional implications for the whole

genome duplications – the retained duplicates were strongly enriched for signaling and regulatory genes (104), a finding that was subsequently confirmed in other organisms (e.g., 71). More recently, gene duplicates have been analyzed in the context of functional genomics data. For example, statistical analysis of the gene expression differences (110), protein interactions (89, 40), protein localization (73, 94), post-translational modifications (2, 32) and other data types (108) comparing duplicates and singletons illustrate the ways that gene function can change after gene duplication. Given the wealth of evidence for functional changes after gene duplication, the current challenge is to systematically identify the specific genetic changes (in the DNA and proteins) that are responsible for these functional changes, providing a mechanistic picture of evolution (discussed below).

Gene order evolution

The comparison of yeast genomes revealed unexpected patterns of gene order (reviewed by 50, 106). Whether under the influence of selection or neutral forces, gene order does not appear to be completely random in yeast genomes. Production of toxic intermediate compounds was measured in genes that are both chromosomal and metabolic neighbours (76), perhaps consistent with selection for either coordinated activity or reduced chance for independent loss of one gene. Evidence for the importance of synteny in fungal genomes has previously been found in the emergence of the *DAL* and *GAL* gene clusters (106, 122), and toxic intermediates could provide a selective explanation for the conservation of metabolic gene order. This remains an area of debate, however, as direct fitness measurements revealed that disrupting the continuity of *GAL* clusters does not necessarily confer lower fitness (63).

Some global correlates for gene order evolution have also been found. Evidence for the co-evolution of gene order and recombination rate has been found in essential gene clusters whose location corresponds to areas of low recombination (82). Further, highly expressed gene pairs in a comparison between *S. cerevisiae* and *C. albicans* were found in close proximity at more than twice the average rate (51).

Population genomics

The similar appearance and general attributes of hemiascomycetous yeasts can be misleading given their underlying genetic variation. Yeast has increasingly been recognized as a powerful model system for population genetics (44, 62). As the cost and ease of genome sequencing and assembly have become ever more favourable, it is possible to sequence complete genomes of individual yeast strains. In light of this, "population genomics" has emerged.

Several studies compared genomes of individual strains (e.g. 25, 46, 97, 102) and characterized population genetic features of yeasts at the genome-wide scale. In one comprehensive study, population structure of *S. cerevisiae* was compared with its wild relative *S. paradoxus* (69). Genomes of 70 geographically diverse isolates from each species revealed that *S. paradoxus* isolates varied along geographic boundaries while *S. cerevisiae* were not nearly as clearly delineated. Geographic variation similar to *S. paradoxus* was reported for another related yeast, *S. kudriavzevii* (46). This, in combination with evidence of more recombination and more phenotypic variation in all lineages of *S. cerevisiae* pointed to the long history of domestication and opportunities for cross-breeding within the species (69).

Population sequence data has greatly facilitated QTL mapping in yeast (reviewed in 10, 28, 87). In addition to traditional quantitative traits (such as growth rates and cell shapes) the molecular tools in yeast expanded the scope of traits that could be associated with genetic variation. Natural variation in genome-wide gene expression profiles was treated as a quantitative trait (11), revealing widespread evidence for complex inheritance amongst most expression levels. These so-called "eQTL" studies in yeast paved the way for similar studies in humans, mice, and plants (56, 77, 103), and widened our understanding of complex inheritance and its evolution in eukaryotes. These approaches have recently been extended to genetic analysis of other types of functional genomics data, such as genome-wide transcription factor binding ("bQTL") and protein concentration traits ("pQTL") (125, 1). Another important area in population genomics is genome-wide association studies (GWAS). While complex population structure in yeast leads to challenges in this area, these are currently being addressed (20, 23).

Mapping the mechanisms of evolution and measuring fitness directly

It has become increasingly possible to reconstruct evolutionary history at the molecular level and to infer the corresponding changes in cellular function and physiology. This so-called "functional synthesis" (22) holds great appeal to evolutionary biologists who have been historically limited to correlative experiments and statistical inference. Yeast is an ideal organism for mechanistic evolutionary experiments for several reasons. First, the largely tree-like evolution of yeast genes (96) and bioinformatic resources (described above) allows molecular history to be reconstructed accurately. For example, reconstructed ancestral maltases from a large gene family show evidence for multiple mechanisms of diversification, including natural selection on key residues that control substrate specificity (114).

Perhaps more importantly, yeast evolution is experimentally accessible. For example, cryogenic preservation of intermediate genotypes creates a living record of the dynamic evolutionary process (13). In addition, the short generation time of yeast enables techniques for systematic, quantitative measurements of fitness (12) and genotype frequencies (39, 88), allowing systematic investigation of evolutionary properties that have been discussed extensively in abstract, but have been hard to measure. For example, repeated adaptation of lab populations revealed direct evidence for pervasive genetic hitch-hiking, a long-predicted feature of natural selection (64), and dramatic losses of environmental response signaling networks when the environment was held constant, revealing a mechanistic explanation for long-discussed evolutionary trade-offs (60). By measuring the fitness of the deletion collection in multiple environments it is possible to systematically identify such genetic trade-offs (antagonistic pleiotropy, 92). Another long-standing question in molecular evolution is the distribution of fitness effects of new mutations, and using engineered libraries this can now be measured directly (42). These tools will also allow for experimental tests of models of adaptation (41).

Thus, it is now possible in yeast to (i) use ancestral reconstruction to infer specific molecular changes, (ii) test functional impacts on protein function and cellular traits, and (iii) measure whether those changes lead to fitness advantages (at least in the environments that are possible to simulate in the lab). This implies the prospect of discovering (after centuries of speculation) how evolution actually happened (22). One of the first and most compelling studies to use direct fitness measurements of re-engineered evolutionary changes was a study of the gene duplication of *GAL1* and *GAL3* within the classical *GAL* regulatory network (45),

which showed direct evidence for fitness increases after gene duplication, consistent with "escape from adaptive conflict" (49). Experimental fitness measurements were also used to provide direct evidence that gene expression differences in an endocytosis complex (implicated through statistical analysis) in the transition to pathogenicity conferred a growth advantage at high temperature (30). Most recently, ancestral reconstructions were used to identify specific amino acid changes in the paralogous transcription factors Mcm1 and Arg80 that led to subdivision of the ancestral gene function, but also (fascinatingly) to avoid interfering with each other through spurious vestigial interactions (5).

The mechanistic perspective of evolution that is now possible in yeast is still only beginning to take hold. However, the early studies in this area have already demonstrated that it will be possible to directly address fundamental questions about gene duplication and regulatory evolution using these approaches.

Evolution of complex phenotypes – the future

Experimental evolution, complemented by next-generation sequencing, has similarly been useful in understanding how complex phenotypes evolve. *S. cerevisiae*, while commonly unicellular, is known to have the ability to form clusters by flocculation and in nature exhibits pseudohyphal growth under starvation conditions. The discovery and characterization of the evolution of different paths to multicellularity in *cerevisiae* (57, 81,95)illustrates the power of *cerevisiae* for studies of evolution of complexity. *S. cerevisiae* evolves a distinct type of multicellularity within sixty days (or less than 500 generations) of applying selection for fast sedimentation (95). These yeast (dubbed "snowflake yeast") do not separate after budding, exhibit distinct juvenile/adult life stages and contain cells that undergo "altruistic" apoptosis to benefit the "organism" as a whole. A similar phenotype has since been evolved and attributed to a frame-shift mutation in the *ACE2* gene (81).

Further examples of the evolution of phenotypic complexity in budding yeasts include insights into the evolution and maintenance of sex (37, 43), cooperation (70, 107, 113), and so-called "cross-protection" (anticipation of future environmental change following periodic environmental shifts) (24). Mechanisms for other complex phenotypes such as "memory" (15) are now being characterized in yeast, and it will be very interesting to see how these have evolved. In conjunction with the experimental evolution approach, the power of yeast genetics is being further exploited in synthetic biology experiments (58, 105). Reconstructing strains that have been evolved experimentally is helpful in determining causation, which we anticipate will be even more important when exploring the evolution of complex phenotypes.

The relative ease with which yeasts can be genetically manipulated combined with any of the abovementioned qualities make budding yeast and its relatives ideal organisms in which to explore key evolutionary mechanisms in detail. Perhaps most surprisingly, yeast has proven an excellent model for studying the evolution of complex phenotypes. It will be especially exciting to watch this area of molecular evolution develop as scientists discover more interesting phenotypes that can be evolved and characterized in the lab.

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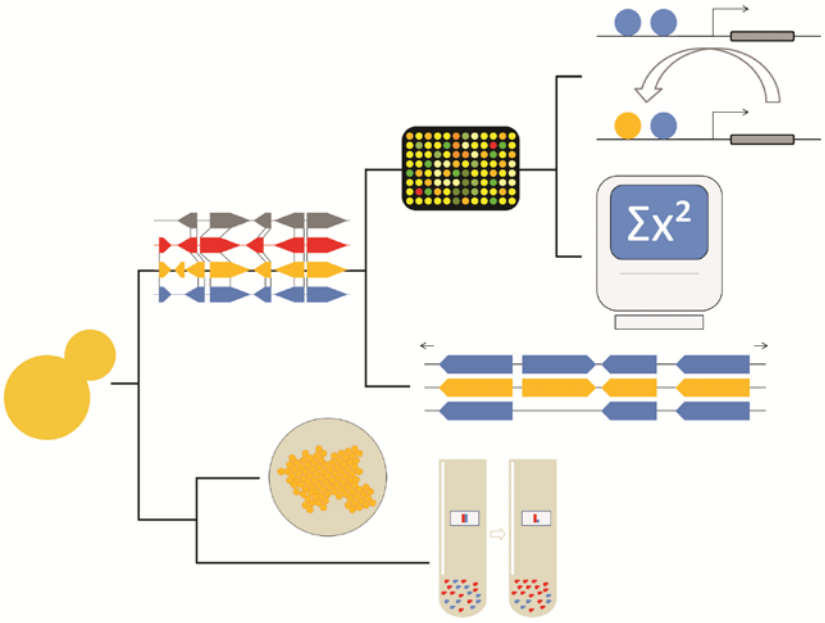

This figure shows the evolution of several of the topics discussed in our review.

Graphical abstract

A schematic representation of the topics we cover in this review. Budding yeast is the "root" of the illustrative phylogenetic tree. Comparative genomics and systematic data collection have provided insights into regulatory network evolution and enabled statistical analysis of key questions in molecular evolution. Availability of comparative genomics data has also catalyzed studies of gene order evolution and gene duplication and divergence. Yeast allows laboratory assays for fitness and serves as a model for experimental evolution of complex phenotypes.