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Exploring conditional gene essentiality through systems genetics approaches in yeast

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An essential gene encodes for a cellular function that is required for viability. Although viability is a straightforward phenotype to analyze in yeast, defining a gene as essential is not always trivial. Gene essentiality has generally been studied in specific laboratory strains and under standard growth conditions, however, essentiality can vary across species, strains, and environments. Recent systematic studies of gene essentiality revealed that two sets of essential genes exist: core essential genes that are always required for viability and conditional essential genes that vary in essentiality in different genetic and environmental contexts. Here, we review recent advances made in the systematic analysis of gene essentiality in yeast and discuss the properties that distinguish core from context-dependent essential genes.

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Introduction

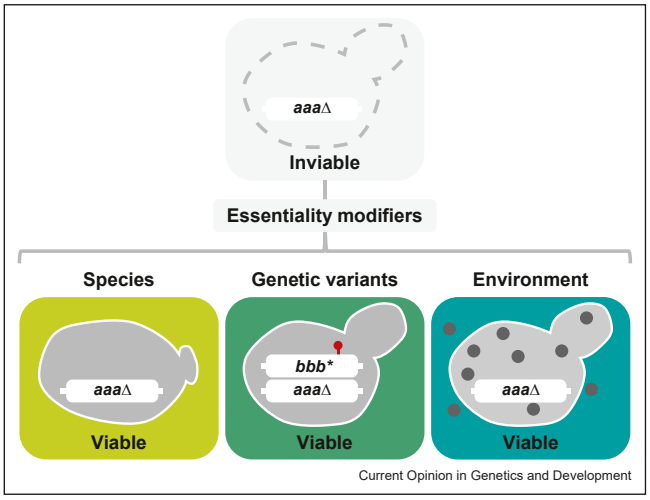
A gene is defined as essential when loss of its function leads to death of a cell or organism. Cataloging which genes are essential is critical to understanding how a cell functions and what the minimal components are that it needs to survive. Gene essentiality has been extensively studied in the budding yeast *Saccharomyces cerevisiae* due to its compact and highly annotated genome and the early availability of efficient genome engineering tools. By systematically deleting each of the ~6000 yeast genes individually, Giaever et al. showed that ~18% of yeast genes are essential for viability under nutrient-rich conditions in the laboratory strain S288c [1], confirming the percentage of essential genes predicted by earlier studies [2,3]. These and other studies showed that essential genes tend to be highly conserved across species, are less likely to have a paralog, are enriched for genes involved in core cellular processes and genes encoding protein complex members, and tend to be more highly expressed compared with nonessential genes [1,4–7].

Despite the critical roles essential genes play in a cell, cellular processes can sometimes be rewired to bypass the requirement for otherwise essential genes. In recent years, systematic studies of gene essentiality have invariably shown that the essentiality of a gene can change between contexts (Figure 1). For example, comparing essential gene sets among yeast species revealed species-specific essential genes [4,6,8–10]. Furthermore, even within the same yeast strain, certain genomic variants were found to give rise to differences in gene essentiality [7,11–15]. Finally, the exposure of cells to particular environments could also modulate gene essentiality [1,16–20]. Understanding how gene essentiality varies between species, genetic backgrounds, and environments is critical for understanding selective pressures during evolution and may aid the identification of efficient antifungal drug targets as well as drug-resistance mechanisms. Here, we review how systems genetics approaches have identified conditional essential genes and their properties, highlight how genetic and environmental contexts influence gene essentiality, and discuss the underlying causes of variation in gene essentiality.

High-throughput approaches for studying gene essentiality

Conventional methods for studying gene essentiality are based on analyzing the viability of haploid meiotic segregants derived from a heterozygous diploid mutant strain [1,3,11,13]. Because haploid essential gene deletion mutants are not viable, new segregants have to be isolated each time viability is tested in different experimental contexts. To avoid this labor-intensive process, we recently generated a haploid conditional deletion mutant collection of essential genes, in which each strain is deleted for an essential gene, but viable because a temperature-sensitive allele of the same essential gene is present on a counterselectable plasmid [7]. In the presence of the plasmid, the strains behave as partial loss-of-function mutants of the essential gene, whereas counterselection against the plasmid enables testing for viability in the absence of the essential gene,

Figure 1



Overview of the main modifiers of gene essentiality. A gene that is essential in a specific strain and condition can be nonessential in other species or become dispensable due to modifications of the genetic background or environment. *aaa*Δ, deletion allele of gene *AAA*; *bbb**, mutant allele of gene *BBB*.

for example, in different genetic or environmental contexts [7].

Another strategy to systematically study gene essentiality is to use CRISPR–Cas9-based screens, which work especially well in yeast species with low rates of homologous recombination such as *Yarrowia lipolytica* [21]. CRISPR–Cas9 screens rely on the generation of DNA double-strand breaks at desired genomic loci. Inaccurate repair of the DNA breaks can lead to basepair insertions or deletions that, when occurring in genes, can generate loss-of-function alleles [21]. In systematic genome-wide CRISPR–Cas9 screens, essential genes can be detected by sequencing pools of mutant cells at different time points and identifying the CRISPR components, and thus indirectly the mutants created by them, that disappear from the population over time. CRISPR–Cas9 systems have also been adapted to disrupt open reading frames (ORFs) by introducing premature stop codons at defined locations in a high-throughput manner [22]. Furthermore, transposon-based mutagenesis screens, which involve high-throughput gene disruption by random integration of transposable DNA sequences into the genome, have been used to induce gene loss-of-function [6,8–10,18,23–25]. By mapping the transposon insertion sites in pools of hundreds of millions of cells, essential genes can be distinguished from non-essential ones based on the insertion density per gene. These screens not only provide information about gene essentiality, but at a high insertion density can also identify particular domains or regions of a gene that are (non)essential [24].

Finally, various types of partial loss-of-function alleles have been developed for essential genes, including temperature-sensitive mutants [26,27] and DAmP alleles [28], as well as several methods to control gene expression such as CRISPRi [29,30] and regulatable promoters [31,32]. However, these are not suitable for the identification of changes in gene essentiality, because partial loss-of-function alleles can often be rescued by mechanisms that cannot overcome the lethality of essential gene deletion mutants [33,34].

Differential gene dispensability in evolutionary distant yeast species

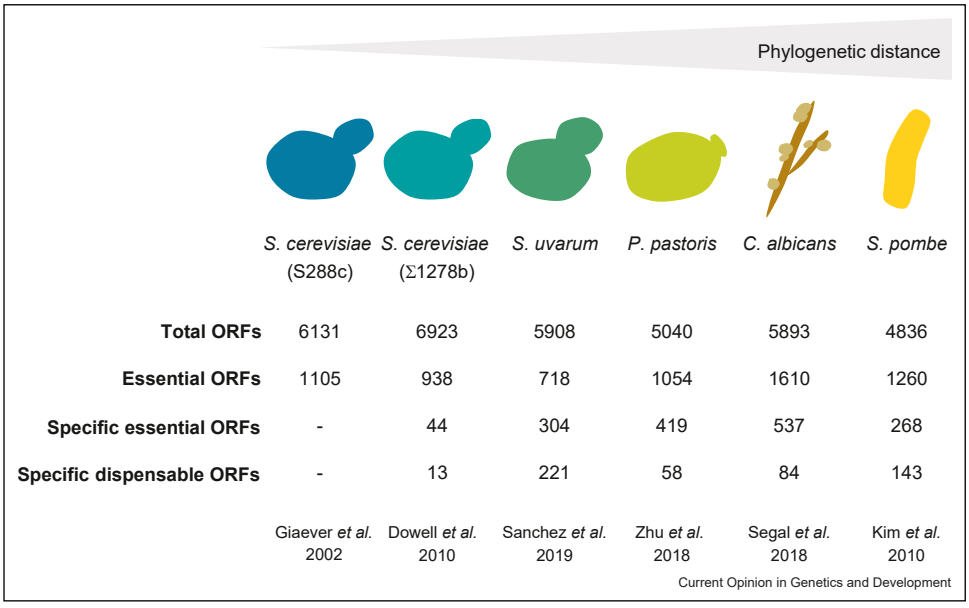
Gene essentiality has been systematically studied for different yeast species, including *S. cerevisiae* [1], *Schizosaccharomyces pombe* [4,8], *Saccharomyces uvarum* [9], *Pichia pastoris* [10], and *Candida albicans* [6]. Comparing gene dispensability between species revealed a surprisingly consistent percentage of essential genes per genome (~15–25% of all genes). The presence of paralogs can mask essentiality of a gene and more compact genomes with fewer redundant genes were thus expected to contain a higher proportion of irreplaceable genes. However, the percentage of essential genes was not found to correlate with the total number of ORFs [4,9] (Figure 2).

Further comparison of the identified essential gene sets revealed a core set of essential genes with roles in fundamental biological processes that are essential among all tested yeast species. These include genes encoding proteins with a role in DNA replication and members of the general transcription factors, the exosome, and other basic cellular machinery. Each species also contains ~300–500 essential genes that are not essential in *S. cerevisiae* [4,6,9,10] (Figure 2). The protein products of these species-specific essential genes are often involved in signaling, transport, metabolism, or regulatory processes [4,6,9]. For instance, genes that are essential specifically in *S. pombe* are highly enriched for mitochondrial genes, as loss of mitochondrial DNA is lethal in fission yeast, while tolerated in budding yeast [4]. Furthermore, although genome size did not affect the overall percentage of essential genes, the presence of gene paralogs can cause individual changes in gene essentiality between species. For instance, *CDC25*, encoding a nucleotide exchange factor that activates Ras, is essential in *S. cerevisiae* but not in *S. uvarum*, which contains the *CDC25* paralog *SDC25* that is nonfunctional in *S. cerevisiae* [9].

Differential gene dispensability in isolates from the same species: *S. cerevisiae*

Changes in gene essentiality are not solely restricted to distantly related species, but can also be observed among strains of the same species. A comparison of gene essentiality in the closely related *S. cerevisiae* strains

Figure 2



Comparison of gene essentiality across systematically studied yeast species [4,6,9–11]. The two bottom rows list the number of genes that, when compared with their orthologs in *S. cerevisiae* strain S288c, are either only essential in the indicated strain or species ("specific essential ORFs") or only essential in S288c ("specific dispensable ORFs"). Note that experimental procedures and orthology mapping methods vary between studies and may affect the identified number of strain-specific or species-specific genes.

S288c and Σ1278b found that 1% of all genes were essential in only one of the two strains [11] (Figure 2). Furthermore, whole-genome sequencing of 1011 *S. cerevisiae* isolates revealed a set of 2856 variable ORFs that were absent in at least one of the genomes [12]. Remarkably, 123 of these variable genes are essential in the S288c background. The presence of paralogs in some isolates, but not in S288c, could explain differences in essentiality for 71 genes. For the remaining 52 differential essential genes, the causes underlying the variation in their dispensability remain unclear [12]. Thus, for a substantial fraction of "essential" budding yeast genes, their requirement is highly dependent on the genetic background.

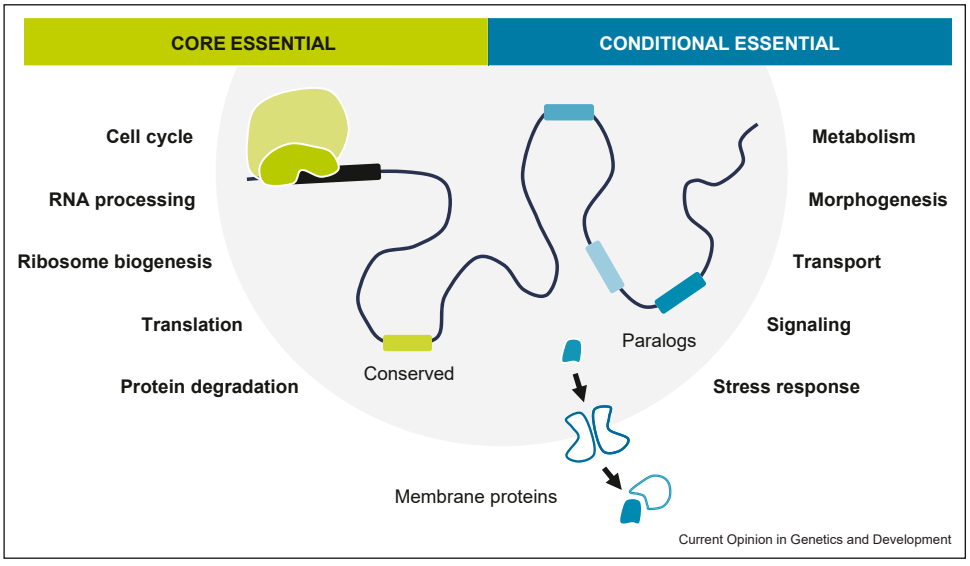
Variation in gene essentiality within the same genetic background

In addition to variation in gene dispensability between different species or isolates, gene essentiality can also vary within the same strain background. For example, nonessential genes can become essential through synthetic lethal interactions, in which the simultaneous perturbation of two nonessential genes leads to cell death. Around half of the nonessential genes in *S. cerevisiae* are essential in the presence of a specific gene deletion mutant [26], suggesting that their requirement for viability is masked by the presence of other genes [35]. Vice versa, well-characterized essential genes can become nonessential in the presence of bypass-suppressor mutations in other genes, revealing a pool of "context-dependent" essential genes [7,13–15,36–38].

Bypass suppression of essential genes is relatively frequent in yeast. In a systematic screen, we showed that in the *S. cerevisiae* laboratory strain S288c, the requirement for ~17% of essential genes (124 out of 728 tested genes) could be bypassed by spontaneous mutations elsewhere in the genome [7]. About half of the bypass-suppressor mutations were single-nucleotide variants, whereas the other half were aneuploidies. Similarly, a study that focused solely on suppression by aneuploidies, found that ~9% of essential genes in S288c could be bypassed by an aneuploidy [13]. By contrast, in *S. pombe*, ~27% of the 92 investigated essential genes could be bypassed, almost twice as much as in *S. cerevisiae* [15]. It is difficult to assess whether this difference in the percentage of bypassed essential genes reflects a true biological phenomenon or if it is caused by the different techniques used to isolate suppressors (chemical mutagenesis, transposon insertions, and artificial gene overexpression in *S. pombe*, compared with spontaneous mutation isolation in *S. cerevisiae*).

In all three studies, context-dependent essential genes more closely resembled nonessential genes than core essential genes, as they tended to be less conserved, enriched for genes with paralogs, and depleted for genes encoding members of protein complexes [7,13,15]

Figure 3



Properties of core and conditional essential genes. Core essential genes (left) are depleted for genes with paralogs (depicted by a single green gene) and involved in core cellular processes (exemplified by gene transcription). The main properties of conditional essential genes (right) are shown, such as enrichments for genes with paralogs (depicted by multiple blue genes) and for genes encoding membrane proteins [4,6,7,9,10,12].

(Figure 3). Conditional essential genes were also enriched for genes encoding membrane-associated proteins and for genes with regulatory functions. In contrast, genes involved in core cellular functions such as RNA processing, translation, and protein degradation were unlikely to be bypassed (Figure 3). Interestingly, many of the properties of context-dependent essential genes identified in these studies were shared with genes that show differential essentiality between species. Furthermore, mitochondrial genes not only tended to be species-specific (essential in *S. pombe* but not in *S. cerevisiae*), but were also enriched in the group of genes that can be bypassed in *S. pombe* [15,39]. Thus, genes that can be rescued by spontaneous mutations within a species have similar properties as those that show variation in essentiality across species.

The genetic basis of conditional essentiality

Several studies that systematically compared gene essentiality among closely related yeast isolates have identified modifier loci that alter gene essentiality. Analysis of haploid progeny of *S. cerevisiae* S288c/Σ1278b hybrids containing a deletion of a conditional essential gene (essential in one background but not in the other) revealed that, in most of the cases, dispensability was impacted by the combined effect of multiple loci [11,40]. Less often, 6 out of 20 studied cases, single modifiers were found [40]. For example, *CYS3* and *CYS4*, both encoding enzymes in the cysteine-biosynthesis pathway, were found to be essential in Σ1278b but not in S288c. Furthermore, *CYS3* was also found to be nonessential in the African palm wine strain Y12. Two independent single modifiers of the conditional essentiality of *CYS3* and *CYS4* were identified: *MET1* in Y12 and *OPT1* in Σ1278b, each with roles associated with cellular cysteine physiology. Remarkably, essentiality of *CYS3* could be accurately predicted in other yeast isolates based on the presence of loss-of-function variants in *MET1* or *OPT1* [40]. Knowledge of the molecular mechanisms underlying differences in gene essentiality can thus be used to predict gene dispensability in other yeast strains.

Furthermore, to understand differences in gene essentiality within the same strain background, general properties of bypass-suppressor genes have been defined [7,13,15]. Overall, the majority of bypassed essential genes (70%) could be suppressed by a single suppression mechanism: only mutation of one particular complex, pathway, or gene could rescue the lethality of a given essential gene [7]. Similar to the natural suppressor variants, the bypass suppressors isolated in the laboratory were often functionally related to the deleted essential gene. About 50% of the 283 identified bypass-suppressor genes carried a loss-of-function mutation, ~35% were associated with a gain-of-function phenotype, and the remaining ~15% acted through an unknown mechanism [7]. An example of suppression by gain-of-function mutations involves the bypass of actin-related proteins Arp7 or Arp9, which regulate the activity of the RSC chromatin remodeling complex. The lethality associated with deletion of *ARP7* or *ARP9* could be bypassed by

missense mutations in the catalytic RSC ATPase subunit Sth1 that may increase Sth1 activity in the absence of Arp7 or Arp9 [7]. Gain-of-function effects also included aneuploidies and gene duplication. For instance, deletion of *NUP116*, encoding a nucleoporin component of the central core of the nuclear pore complex, could be suppressed by an increase in copy number of its paralog *NUP100*, either by overexpressing *NUP100* individually or through an extra copy of chromosome XI on which *NUP100* is located [7]. Interestingly, the majority of bypassable essential genes were either always suppressed by an aneuploidy or always by a genomic point mutation, even if both events led to a gain-of-function effect [7]. In the vast majority of the cases, including suppressor aneuploidies, a single gene was responsible for suppression [7,13,15]. This highlights differences between conditional essentiality across diverse genetic backgrounds, which is often driven by multiple modifiers, and suppression within the same strain background, which is mainly driven by a single modifier. A possible explanation for this difference is that the bypass suppressors isolated in a laboratory setting are often not able to restore the fitness of an essential gene deletion strain to wild-type levels and multiple mutations may thus be needed for an essential gene deletion mutant to have a competitive fitness in a natural setting.

Beyond auxotrophy: environment-dependent essential genes

Gene essentiality can also be dependent on the environment. Together with the description of the *S. cerevisiae* deletion mutant collection in 2002, Giaever et al. investigated the fraction of genes necessary for growth in different environments, including changes in carbon source, amino acid availability, osmolarity, salinity, pH, and the presence of antifungal agent nystatin. As expected, genes involved in amino acid biosynthesis were no longer essential when cells were provided with those nutrients. Beyond auxotrophy, this study revealed between ~20 and 120 environment-specific essential genes per condition [1]. More recent systematic studies have investigated genes that sensitize yeast to particular environments, with the aim to expand the yeast interactome [19,41–43], identify new drug targets [44–49], or find genes implicated in the metabolism of certain compounds (e.g., methanol) [10,19,43]. A recent systematic study in *S. cerevisiae* surveying 14 diverse conditions revealed that 59% of tested gene mutants had a growth defect in at least one condition [19]. Although the specific properties of environment-dependent essential genes have not been thoroughly described, environments often affect genes with a close functional relation to the pathways that are perturbed by a condition [19]. For example, genes that displayed a fitness defect in the presence of Monensin, an intracellular traffic inhibitor, often functioned in vesicle trafficking and cell-wall biogenesis [19].

Gene essentiality can also be suppressed by environmental factors and some essential genes can thus become dispensable under nonstandard growth conditions. For instance, deletion mutants of essential genes involved in cell-wall integrity and composition, that normally die of osmotic imbalance due to a lack of cell-wall stability, can be rescued by osmotically stabilized environments in both *S. cerevisiae* and *S. pombe* [16–18,20]. While examples of environmental suppression of essential genes have been described, they are limited to individual genes or conditions. It thus remains unclear what fraction of essential genes can be bypassed by a change in environment.

Conclusion and outlook

The application of systematic genetic approaches to study gene essentiality in yeast has led to the identification of two sets of essential genes: ~560 core essential genes that are always required for viability, independent of the genetic background or environment, and many more context-dependent essential genes that differ in importance among conditions [4,6,7,9,10,12,13]. Despite variation in experimental design, model system, and growth conditions, the conditional essential gene sets share remarkable overlap in genes and functional properties across studies. Whereas core essential genes are generally involved in fundamental cellular functions, such as translation or protein degradation, conditional essential genes tend to be involved in more accessory functions and frequently have paralogs.

Even with the remarkable progress on mapping yeast essentialomes in the past years, several questions remain unanswered. First, differences in gene essentiality have been explored in several yeast species and *S. cerevisiae* strains, but our understanding of how gene essentiality varies throughout the yeast clade remains limited. Second, identification of the genetic factors driving differences in gene essentiality across yeast strains and species is often lacking. Third, the fraction of essential genes that become nonessential under different environmental conditions remains unexplored. Finally, most systematic studies have focused on the essentiality of protein-coding genes in haploid cells. Our understanding of how essentiality varies across contexts during sexual reproduction [50], in diploid cells, for noncoding RNAs [51,52], or for protein isoforms, thus remains incomplete. Systems genetics approaches, possibly combined with machine-learning strategies, will be needed to answer these questions and to achieve a thorough description and interpretation of the yeast essentialome. Knowing how gene essentiality can vary between species, strains or individuals, and environments and understanding the underlying causes will benefit the design of synthetic minimal genomes, aid the development of specific treatments against pathogenic fungi that

have little effect on closely related benign species, highlight antifungal drug-resistance mechanisms, and will provide insight on how genetic variance accumulates during evolution and affects genetic traits.

Conflict of interest

Nothing declared.

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