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Genetic suppression: Extending our knowledge from lab experiments to natural populations

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Summary

Many mutations have deleterious phenotypic effects that can be alleviated by suppressor mutations elsewhere in the genome. High-throughput approaches have facilitated the large-scale identification of these suppressors and have helped shed light on core functional mechanisms that give rise to suppression. Following reports that suppression occurs naturally within species, it is important to determine how our understanding of this phenomenon based on lab experiments extends to genetically diverse natural populations. Although suppression is typically mediated by individual genetic changes in lab experiments, recent studies have shown that suppression in natural populations can involve combinations of genetic variants. This difference in complexity suggests that sets of variants can exhibit similar functional effects to individual suppressors found in lab experiments. In this review, we discuss how characterizing the way in which these variants jointly lead to suppression could provide important insights into the genotype-phenotype map that are relevant to evolution and health.

Keywords

complex traits; epistasis; genetic interaction; genotype-phenotype map; natural genetic variation; positive genetics interactions; suppressors

Introduction

Genetic interactions occur when combinations of mutations show phenotypic effects that differ from expectations based on individual mutations [1–12]. Among the types of genetic interactions that can occur [12–14], suppression represents an extreme case in which one or more genetic changes at other sites in the genome (i.e., ‘suppressors’) reverse a mutation’s deleterious effects [15, 16]. Identifying these suppressors can provide valuable insights into the functional mechanisms by which mutations jointly affect phenotype [16–18]. As we describe below, high-throughput sequencing and genomics strategies have led to new approaches for rapidly identifying mutation-suppressor combinations on a large scale. These methods have helped produce general insights into the mechanisms that can cause suppression within and between genes in lab experiments.

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Suppression has also become a point of interest for researchers focused on understanding how naturally occurring genetic differences among individuals alter the effects of mutations, thereby leading to incomplete penetrance and variable expressivity. For example, recent studies in yeast [19–21] and humans [22, 23] have shown that the ability to suppress particular large effect and Mendelian mutations can segregate within populations. At present, the extent to which lab studies on suppression relate to these cases of naturally occurring suppression is unclear. Notably, while suppression in the lab typically entails one suppressor interacting with a mutation [12], suppression in natural contexts may involve combinations of genetic variants that collectively revert the effect of a mutation (Fig. 1).

In this review, we attempt to broadly synthesize work on suppression and leverage knowledge gained from lab experiments to provide insights into the genetic and molecular basis of suppression in natural populations.

High-throughput techniques for identifying suppressor mutations in lab experiments

High-throughput approaches have facilitated the comprehensive identification of suppressors in experimental systems. In the following section, we summarize these techniques, differentiating between approaches that primarily enable identification of suppressors that occur in the same gene as the mutation whose effect they alleviate (‘intragenic’ suppressors) and suppressors that occur in different genes (‘intergenic’ or ‘extragenic’ suppressors):

Comprehensive mutagenesis of individual genes

Researchers attempting to identify intragenic suppressors can employ site-directed or random mutagenesis to generate a large subset, if not all, of the possible single and double mutants of a given gene [24–27] (Fig. 2A). Comparison of single and double mutant phenotypes can be used to identify cases of intragenic suppression (Fig. 2B). Studies of this type can examine a large fraction of the possible genetic interactions within a given gene, especially if a gene is small. Such projects are presently constrained by the read lengths of short read sequencing technologies and the throughputs and error rates of long read sequencing technologies [28, 29]. However, as long read sequencing technologies increase in throughput and accuracy, larger genes, and potentially even sets of functionally related genes, may become amenable to gene-specific mutagenesis techniques.

Mapping induced or spontaneous suppressors

Suppressors can be obtained by screening for induced or spontaneous mutations that revert the phenotype of the original mutant (Fig. 3A). However, revertants recovered from these screens typically carry multiple mutations and which mutation is the suppressor is not always clear-cut [12, 16]. A straightforward strategy to distinguish a mutation with a phenotypic effect from its co-occurring ‘passenger’ mutations is by using crosses in combination with whole genome sequencing [12, 21]. Recently, such an approach was used to identify more than 200 mutation-suppressor pairs in a single study [12].

Genome-wide knockout screens

Large-scale screens for genetic interactions have been performed by systematically deleting each gene in the genome in a mutant background (Fig. 3B). In yeast for instance, this has been accomplished by crossing strains that carry a query mutation to a genome-wide collection of gene deletion strains to generate every possible double deletion mutant [12,30]. With the availability of CRISPR/Cas9 gene editing technologies [31–33], similar genome-wide genetic interaction screens can now be implemented in other species. A caveat here is that some suppressors are gain-of-function mutations [12,34], which may not be identifiable in a screen focused on gene knockouts.

Genome-wide screens involving overexpression or silencing

Dosage suppression occurs when a mutant phenotype is rescued by overexpression of another gene [35] (Fig. 3C). High-copy plasmid libraries have been used to successfully identify dosage suppressors [35, 36], and similar screens are now possible using CRISPR/Cas9 activation (CRISPRa) [37–39]. Much like overexpression screens, genes that act as suppressors when they are downregulated can also be identified using approaches that instead repress transcription at interacting genes using RNA interference (RNAi) [40, 41] or CRISPR/Cas9 interference (CRISPRi) [42–45].

In the next section, we discuss some of the general insights that have been gained from studies of genetic suppression to date.

Functional mechanisms that cause genetic suppression

Past studies, including recent work using the approaches mentioned above, have described a number of mechanisms that can lead to genetic suppression [12, 17, 35, 46]. These mechanisms include:

Reverting a mutated amino acid

Intragenic suppressors in coding regions may change a mutated codon so that it specifies an amino acid that is structurally or biochemically similar to the one that was initially present [17]. Intergenic suppressors in tRNAs can have a similar effect by changing the codons that specify a particular amino acid [16].

Restoring the structural conformation or dosage of an mRNA, protein, protein complex, or cellular component

Intragenic suppressors can impact transcript or protein stability, thereby allowing a mutated gene to function at closer to wild type levels [17, 46]. Intergenic suppressors might affect the physical interaction between proteins by altering sites of protein-protein interaction [47], increasing the levels of available binding partners [35], or even enabling a protein complex or cellular component to function in the absence of the originally mutated gene's protein product [48].

Changing the dosage of a mutated gene's cognate mRNA or protein

Both intragenic and intergenic suppressors may directly alter the levels of a mutated gene's cognate mRNA or protein [12, 17, 35, 46]. Changing dosage in this way may compensate for the reduced activity of a gene product due to the destabilizing effect of an initial mutation. Intragenic suppressors that act in this way may occur in *cis* regulatory elements, whereas intergenic suppressors could occur in transcription factors and their regulators [16].

Modified activity within a pathway

An intergenic suppressor may occur in a gene that is in the same pathway as the original mutation, thereby restoring wild type activity levels within that pathway [12]. For instance, one gene may activate downstream targets of the pathway, while the other represses these targets. A mutation in the activator that disrupts the balance between these regulators could inactivate the pathway. Loss-of-function in the corresponding repressor or gain-of-function in a parallel activator in the same pathway could then suppress the effect of the first mutation and restore the pathway's function.

Changes in activity between pathways

Intergenic suppressors can also occur in pathways that perform functions related to that of the pathway containing the original mutation, which may be able to functionally compensate for altered activity in the initial mutant. However, research suggests this form of genetic suppression is less prevalent than some of the other mechanisms described above [12, 30, 49]. This could in part be due to the negative effects of network rewiring, as the gain-of-function mutation in the suppressor allele may disrupt the original function of the gene harboring the suppressor, resulting in a negative fitness effect.

Global changes in transcription, translation, or other cellular processes

Suppressors may act in a more general manner by influencing overall levels of transcription and translation in cells [12]. For example, a mutation that reduces the expression of a gene might be suppressed by another mutation that decreases protein degradation.

As described in this section, lab experiments have been used to comprehensively determine the mechanisms that can give rise to genetic suppression. Information from these experiments is a valuable research tool for considering how genetic suppression might occur in other contexts, such as in natural populations.

Examples of genetic suppression in natural populations

Although genetic suppression has historically been a focal point for researchers interested in dissecting pathways and genetic networks [12], it is now becoming increasingly important to scientists who study heritable phenotypic variation within natural populations. Multiple examples of suppression have been identified in different species, particularly yeast [19, 20] and humans [22]. Such naturally occurring suppression might play an important role in evolution and disease.

One of the most striking examples of naturally occurring suppression comes from genes that are essential in only certain individuals within a species. Essential genes encode fundamental cellular functions that are required for viability. However, which genes are essential varies from individual to individual because of differences in their genetic backgrounds. To demonstrate this point, Dowell et al. knocked out nearly all of the roughly 5,000 genes in two strains of the budding yeast *Saccharomyces cerevisiae* [19]. By doing this, they found 57 genes that were essential in only one strain or the other. This represents nearly 6% of the genes that were essential in either of the strains.

In another study focused on yeast, Hou et al. found that naturally occurring suppressors can have a large effect on the viability of segregants in *S. cerevisiae* crosses [20]. Specifically, they identified a genetic variant in a tyrosine tRNA that allows read-through of TGA stop codons. This tRNA variant suppresses a nonsense allele in a mitochondrial cytochrome c-oxidase (*COX15*), thereby reverting the respiratory defect shown by individuals with the *COX15* nonsense allele.

Large-scale whole genome resequencing studies in humans have found similar results to the work in yeast. For example, an analysis of ~590,000 genomes identified 13 adults who were healthy even though they possessed disease-associated genotypes at fully penetrant early onset Mendelian disease loci [22]. In another study, sequencing of exomes from 3,222 highly related individuals identified 1,111 homozygous variants with predicted loss of function in 781 genes [50]. Despite the fact that some of the variants had previously been associated with diseases, no significant correlation was observed between an individual's genotype and health record [50]. Resequencing studies have shown that other species also large amounts of rare loss-of-function variation in phenotypically important genes (e.g., [51–53]).

Additionally, Jordan et al. used comparative genomics to discover intragenic suppressors of human disease mutations that were present in other species [23]. They first characterized the extent to which disease alleles have fixed in 100 non-human vertebrates. Up to 12% of the queried variants showed fixation in at least one non-human genome, suggesting that other genetic differences in these outgroups ameliorated the effects of the disease alleles. Examination of orthologous amino acid sequences using a computational model facilitated the identification of potential intragenic suppressors, several of which were experimentally validated.

These findings in yeast, humans, and other species reflect a broader reality that genetic background often plays a strong role in influencing how large effect mutations impact phenotype [54,55]. In some cases, alleles that appear to have Mendelian effects in some genetic backgrounds can show more complicated phenotypic impacts when other backgrounds are considered [56]. Also, some mutations may affect viability in a more probabilistic manner, with different genotypes varying in their propensities to survive a particular genetic perturbation. For example, Paaby et al. found extensive genetic variation in embryonic lethality among *Caenorhabditis elegans* strains by using RNAi knockdown of important developmental regulators [57].

Following upon the results in this section, as well as similar studies that were not discussed, it is important to determine the genetic and molecular mechanisms that cause individuals to show different responses to the same large effect mutations.

The genetic and molecular basis of naturally occurring genetic suppression

Characterizing the mechanisms that underlie naturally occurring suppression can provide novel insights into how genetic variation within populations can modify the responses of individuals to new mutations. As a starting point for considering this problem, one must appreciate that populations often harbor large amounts of genetic variation, which can rewire the pathways and networks that give rise to phenotype [58–65]. These changes can cause individuals to differ in the genes they require to be healthy or viable, or to express a given trait.

As with most traits that segregate within species, suppression in natural populations may have a complex genetic basis that involves multiple variants (Fig. 1). Supporting this view, crossing experiments in budding yeast indicate that most conditional essentialities are mediated by two or more genetic variants [19, 66]. More generally, work on other cases in which a mutation shows different effects across genetic backgrounds has shown that response to mutations can be mediated by higher-order sets of variants that interact not only with a mutation, but also with each other [21, 64, 67–70] and potentially even the environment [68]. However, only a small number of examples of this phenomenon have been comprehensively teased apart at the genetic level [55], leaving open the possibility that other genetic architectures, such as those in which a mutation acts as a hub of pairwise genetic interactions with many different variants [7, 8], could also be important.

Moving forward, it is necessary to better determine the genetic and molecular basis of suppression in natural populations. Such work could be difficult in humans, but is feasible in model systems that facilitate comprehensive genetic dissection of complex traits [71,72]. Research along these lines can answer questions about the number and molecular functions of involved genes and genetic variants, and can clarify the relationship between the functional mechanisms of suppression in lab experiments and natural populations.

Given the genetic complexity that can underlie suppression in natural populations, intergenic suppressors may be more likely to contribute in nature than intragenic suppressors because of their significantly larger target space for accumulating genetic variation. Beyond this distinction, categorizing the functional mechanisms that are responsible may not be as straightforward as in lab experiments. Naturally occurring suppression might involve a number of variants with small effects on molecular function, which may individually alter the structures of mRNAs and peptides, enzymatic activities, or transcript and protein levels in subtle ways (e.g. [21]). Some of these variants may influence individual genes, whereas others may have more global effects, in some cases influencing the expression of a large fraction of the genome. Combinations of these variants could then collectively achieve a similar functional effect to the individual suppressors typically seen in lab experiments (Fig. 4).

Characterizing the genetic and functional mechanisms underlying naturally occurring suppression will provide valuable insights into how combinations of variants can alter the susceptibility of biological systems to genetic perturbations. This problem has a fundamental bearing on our understanding of the ways in which new mutations and pre-existing genetic variation jointly determine the relationship between genotype and phenotype.

Conclusion and outlook

Lab experiments enabled by high-throughput genetic and genomic approaches have helped provide detailed insights into the distinct functional mechanisms that give rise to genetic suppression. Given that suppression appears to segregate within species, determining how findings from the lab relate to natural populations is important. Because of the large amount of genetic variation in these populations, naturally occurring suppression may in some cases be more complex at the genetic and molecular levels than suppression studied in the lab. Work that is able to tease apart this complexity may provide new insights into how genetic variation within species alter the susceptibilities of individuals to large effect mutations.

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Figure 1.

Genetic basis of suppression in lab versus natural environments. **A:** In lab experiments, populations start out as genetically identical and suppressors arise as new single mutations on the background of the original mutation. **B:** Large amounts of genetic variation segregate within natural populations and some of these variants can individually or collectively act as suppressors in a similar fashion to the large-effect mutations identified in lab experiments. In both **A** and **B**, each chromosome represents a haploid individual. Grey chromosomes exhibit the mutant phenotype, while black chromosomes do not. Stars represent a mutation of interest, while vertical bars show other genetic changes in the population. Red vertical bars individually or collectively act as suppressors, while black vertical bars have no effect on the mutant phenotype.

Figure 2.

Identifying intragenic interactions across an entire gene. **A:** Using directed or random mutagenesis, a library of mutants can be constructed such that every pairwise (or potentially higher-order) combination of nucleotides in a gene (*YFG*– ‘your favorite gene’) is mutated. **B:** Each single and double mutant can be examined in order to identify all of the potential genetic interactions that can occur within a gene. Colors refer to the difference between the observed fitness of each double mutant minus the expected value given the fitnesses of the two single mutants. Instances of suppression may be observed if the fitness of a double mutant is higher than expected.

Figure 3.

Techniques for identifying intergenic suppressors. Multiple types of genetic screens have been utilized to identify suppressors in lab experiments. **A:** New mutations generated either through mutagenesis or spontaneous mutation can result in suppressors (red). This approach usually results in the identification of multiple mutations, from which the actual suppressor must be distinguished. **B:** Genome-wide suppressor screens can be accomplished by systematically knocking out each gene in a mutant background. This can be accomplished by targeted gene deletion or recombination with a collection of knockout strains. **C:** Dosage suppression interactions can be screened using different strategies for altering the regulation of each gene in the genome using either plasmid-based overexpression, RNAi, or CRISPRa/CRISPRi.

Figure 4.

Combinations of genetic variants may cause suppression in natural populations. **A:** A signaling network may regulate the expression of a transcript that is required for viability. **B:** Knockdown of a key component of the network (red) can result in loss of a required transcript. **C:** As is typically observed in lab experiments, a large effect mutation (blue) elsewhere in the pathway can suppress the deleterious effects of the initial mutation and restore activity. **D:** In natural populations, multiple variants with small functional effects within the pathway (light blue) can collectively result in suppression if their combined effect on network activity is sufficient to achieve levels of pathway output required for viability.