Diverse Molecular Mechanisms Underlying Pathogenic Protein Mutations

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Diverse Molecular Mechanisms Underlying Pathogenic Protein Mutations: Beyond the Loss-of-Function Paradigm

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Abstract

Most known disease-causing mutations occur in protein-coding regions of DNA. While some of these involve a loss of protein function (e.g., through premature stop codons or missense changes that destabilize protein folding), many act via alternative molecular mechanisms and have dominant-negative or gain-of-function effects. In nearly all cases, these non-loss-of-function mutations can be understood by considering interactions of the wild-type and mutant protein with other molecules, such as proteins, nucleic acids, or small ligands and substrates. Here, we review the diverse molecular mechanisms by which pathogenic mutations can have non-loss-of-function effects, including by disrupting interactions, increasing binding affinity, changing binding specificity, causing assembly-mediated dominant-negative and dominant-positive effects, creating novel interactions, and promoting aggregation and phase separation. We believe that increased awareness of these diverse molecular disease mechanisms will lead to improved diagnosis (and ultimately treatment) of human genetic disorders.

INTRODUCTION

Remarkable advances in high-throughput sequencing over the past two decades have improved the outlook for diagnosing human monogenic genetic disease. Clinical sequencing is becoming routine for a wide variety of human genetic disorders, and large-scale research cohorts are efficiently identifying many new pathogenic variants (71, 78). Despite this, most identified variants are still of uncertain clinical significance, and there are still many disorders for which causative mutations are unknown (129). Furthermore, even when pathogenic mutations have been identified, the mechanisms by which they cause disease are often unclear. A better understanding of molecular disease mechanisms has the potential to significantly improve both the diagnosis and treatment of human genetic disorders.

Most known disease-causing mutations affect protein-coding regions of DNA (109). Therefore, considering the protein-level effects of mutations can be extremely valuable for identifying pathogenic mutations and understanding the mechanisms by which they cause disease. The specific molecular mechanisms by which protein mutations cause disease are diverse but can be classified into three general categories depending on their consequences at the protein level:

- Loss-of-function mutations involve a loss of the normal biological function of a protein. Often these are nonsense or frameshift mutations that introduce premature stop codons. Due to nonsense-mediated decay of the resulting mRNAs, most premature stop codons will result in no protein being produced, rather than a truncated protein (22). However, there are also many examples of loss-of-function mutations that change the amino acid sequence and result in nonfunctional protein products. These mutations can cause a complete loss of function (amorphic), analogous to a protein null mutation, or only a partial loss of function (hypomorphic).
- Gain-of-function mutations have their phenotypic effect because the mutant protein does something different than the wild-type protein. Often, these mutations cause disease by increasing protein activity (hypermorphic) or introducing a completely new function (neomorphic), but as we will see, the specific molecular mechanisms underlying gain-of-function mutations can be complex.
- Dominant-negative mutations involve the mutant protein directly or indirectly blocking the normal biological function of the wild-type protein (antimorphic). They can thus cause a disproportionate (>50%) loss of function, even though only half of the protein is mutated.

When we discuss loss-of-function versus gain-of-function versus dominant-negative mutations in this review, we are focused on the effects of mutations at the protein level. Occasionally there is ambiguity in the literature when referring to these phenomena. For example, disruption of an inhibitory protein leading to enhancement of a specific biological activity could in some instances be referred to as a gain of function (88). However, according to our definitions, this is a loss of function at the protein level, as the protein has lost its activity. Thus, a protein null mutation will always be a loss of function. For a mutation that changes the coding sequence, the ultimate test of whether it is a loss of function is whether or not the phenotypic effect is equivalent to reducing or abolishing protein expression.

The inheritance pattern of a mutation is often a useful indicator of the molecular disease mechanism. If a disorder shows recessive inheritance, it is almost always due to loss-of-function mutations. However, determining the molecular mechanisms underlying dominant disorders is much more difficult. There are also many heterozygous loss-of-function mutations that cause disease, often referred to as haploinsufficient or dosage-sensitive mutations. The vast majority of gain-of-function mutations will be dominant, although there are some rare examples of recessive gain of function (21, 35, 128). Finally, dominant-negative mutations are dominant by definition.

A powerful way to understand the molecular effects of pathogenic mutations is to consider them from a protein structural perspective. Given the massive proliferation of experimentally determined protein structures (18), as well as recent dramatic improvements in our ability to computationally predict structures (7, 63), the utility of structure-based analyses has grown tremendously. However, the structural interpretation of disease mutations has been dominated by a line of thinking that we refer to as the loss-of-function paradigm, which assumes that most pathogenic mutations cause a loss of function by disrupting protein structure and, similarly, that mutations that are disruptive to protein structure will cause a loss of function. While many mutations do cause a simple loss of function, often by destabilizing proteins and preventing them from folding correctly (41, 108, 110, 135), there are a large number of exceptions.

Recent work suggests that non-loss-of-function mutations tend to be poorly identified by computational variant effect predictors (43). In part, this can be explained by the fact that dominant-negative and gain-of-function mutations usually involve milder substitutions between more similar amino acids and are therefore less disruptive to protein structure than loss-of-function mutations, which makes it harder to distinguish them from benign variants observed in the human population. However, this underperformance was even observed for predictors relying solely on evolutionary conservation. As variant effect predictors are widely used in current sequence analysis and interpretation pipelines to prioritize potentially pathogenic variants, these results suggest that the importance of many non-loss-of-function mutations is likely being missed.

In this review, our objective is to shift the focus away from the loss-of-function paradigm and to highlight the great diversity of molecular mechanisms that underlie human disease mutations. As the effects of nearly all non-loss-of-function mutations can be explained by considering the interactions of proteins with other molecules, we have structured this review in an interaction-centric manner. We discuss the different ways in which intermolecular interactions can be used to understand pathogenic mutations, often via complex and nonintuitive mechanisms.

MUTATIONS THAT DISRUPT INTERACTIONS

Loss of Interactions Causing Loss of Function

Disease-causing mutations are enriched at and near protein–protein, protein–DNA, and protein–ligand interfaces (29, 48, 62, 73, 134). Most of these pathogenic interface mutations will probably disrupt interactions, as it is more likely that a given mutation will disrupt an evolutionarily optimized interface than enhance binding (102). There are many examples of loss-of-function mutations that disrupt interactions with different types of binding partners. For example, several pathogenic mutations in calcium/calmodulin-dependent serine protein kinase (CASK) disrupt interactions with its various binding proteins, such as the postsynaptic scaffold protein Sap97, presynaptic cell adhesion protein, neurexin, and T-box brain protein 1 (91). Loss of these interactions is thought to cause neurodevelopmental disorders by disrupting CASK functions—namely, by causing disrupted targeting of Sap97 to receptors, loss of neurexin-induced oligomerization, and loss of transcriptional regulation.

Mutations that impair binding to nucleic acids can also abolish protein function, resulting in numerous pathologies, including cardiovascular (132), neurological (106), and retinal (24) diseases as well as cancer (113). Notably, phenotypic severity has in some cases been linked to the extent to which the mutation disrupts DNA binding (106).

Loss of function is often observed for pathogenic mutations at sites involved in small-molecule interactions. Several mutations in homogentisate 1,2-dioxygenase (HGD) are predicted to reduce the binding affinity to homogentisic acid, leading to the accumulation of toxic homogentisic acid and resulting in alkaptonuria (95). Multiple mutations in *AIPL1* disrupt interactions with prenyl

groups bound to isoprenylated photoreceptor phosphodiesterase 6 (PDE6) (12, 130). This loss of binding causes Leber congenital amaurosis, a severe form of childhood blindness (46), by preventing the mutant protein from acting as a chaperone for PDE6 (75).

Mutations can also perturb catalytic activity by modifying substrate binding affinity and/or the specific orientation required to favor the formation of subsequent transition states, which accelerates the reaction. For example, pyridoxamine 5′-phosphate oxidase (PNPO) catalyzes the oxidation of pyridoxine 5′-phosphate (PNP) to pyridoxal 5′-phosphate (PLP). It is believed the oxidation occurs by transferring a hydrogen anion from the C4′ of PNP to the cofactor flavin mononucleotide (FMN) (33). A homozygous mutation (R229W) in PNPO alters the active site of the mutant protein so that two essential hydrogen bonds at residues H227 and R225 are disrupted, resulting in a loss of affinity to both the substrate and FMN, as well as reduced catalytic activity (87). This leads to reduced activity of PLP-dependent enzymes, causing neonatal epileptic encephalopathy, which can be fatal but is treatable with PLP (97).

Loss of Interactions Leading to Overall Gain of Function

However, disruption of interactions does not always lead to a loss of function. Some interaction-disrupting mutations result in an overall gain of function. For example, one study found that two mutations (W330R and D333N) in the DNA methyltransferase DNMT3A cause microcephalic dwarfism (56). The wild-type residues, located in the PWWP domain, are crucial for interacting with H3 histones that have been di- and trimethylated at lysine 36 (H3K36me2 and H3K36me3, respectively) by forming an aromatic cage around the methylated lysine. This interaction directs DNMT3A to preferential genomic regions for subsequent methylation. Both mutations impair binding to H3K36me2 and H3K36me3. Surprisingly, this impairment was associated with gain-of-function hypermethylation, because the loss of binding to methylated H3 increases the availability of DNMT3A to interact with other genomic sites that do not contain H3K36me2/3 marks, such as Polycomb-associated regions, which are not normally subject to DNA methylation (**Figure 1**). The authors demonstrated that this hypermethylation led to the switching-off of key developmental genes involved in pluripotency in favor of cell differentiation, which is believed to cause

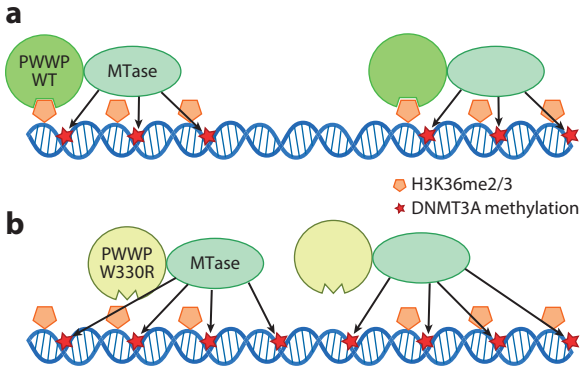


Figure 1

Gain of function caused by a disrupted interaction in DNMT3A. (*a*) Wild-type (WT) DNMT3A is able to bind to histone H3 that is di- or trimethylated at lysine 36 (H3K36me2/3) via its PWWP domain. Thus, its methyltransferase (MTase) domain preferentially methylates DNA in H3K36me2/3-marked regions. (*b*) The pathogenic W330R mutation disrupts the interaction between the PWWP domain and H3K36me2/3, enabling DNMT3A to hypermethylate other regions that do not contain these histone marks, such as Polycomb-associated regions.

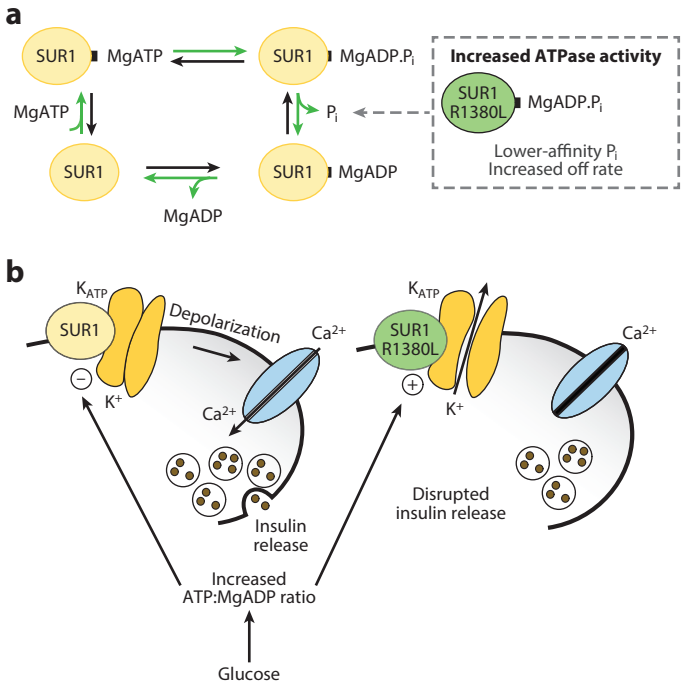


Figure 2

Neonatal diabetes caused by increased ATPase activity in SUR1. (*a*) The SUR1 ATPase catalytic cycle. The R1380L mutant has a lower affinity for inorganic phosphate (P_i), facilitating its release and increasing ATPase activity. (*b*) K_{ATP} channel activity and glucose-stimulated insulin release with wild-type (*left*) and R1380L (*right*) subunits. Insulin release occurs via a calcium influx into the cell, triggered by K_{ATP} channel closure. Glucose increases the intracellular concentration of ATP and reduces that of MgADP. ATP binding to K_{ATP} causes the channel to shut, while MgADP binding to the SUR1 subunit opens K_{ATP}. In normal individuals, the increased ATP:MgADP ratio results in K_{ATP} closing. By contrast, the increased release of P_i in the R1380L mutant increases the SUR1-MgADP bound state, which opens the K_{ATP} channel and disrupts insulin release.

smaller organ size by depleting pools of progenitor cells prematurely, resulting in a reduction in final cell number.

Mutations that disrupt interactions with small molecules can also cause a gain of function by increasing catalytic activity. For instance, *ABCC8* encodes SUR1, a regulatory subunit of the ATP-sensitive potassium channel (K_{ATP}), which acts as a metabolic sensor to allow the K_{ATP} channel to respond to changes in glucose metabolism in pancreatic β-cells. A mutation (R1380L) in SUR1 weakens the interaction with the posthydrolytic phosphate group, increasing the off rate of inorganic phosphate and thereby increasing ATPase activity (30) (**Figure 2*a***). This enhanced ATPase activity increases the probability of the K_{ATP} channel opening, which in turn disrupts the regulation of insulin secretion and causes neonatal diabetes (**Figure 2*b***).

Loss of Interactions Inducing Competitive Dominant-Negative Effects

Mutations that disrupt specific interactions can have dominant-negative effects by competing with wild-type protein. This phenomenon relies on the mutant losing some aspect of its functionality but still being able to bind to something else, thus indirectly affecting the activity of the wild type through competition. For example, the S48F mutation in the PAX8 transcription factor

causes congenital hypothyroidism (47). While the mutation is not destabilizing and does not affect DNA binding, it appears to affect the recruitment of the transcriptional coactivator p300. In a heterozygous state, it is speculated that the S48F mutant will compete with wild-type PAX8 for DNA binding, thus leading to an overall reduction in PAX8 activity and causing disease via a dominant-negative effect.

MUTATIONS THAT INCREASE BINDING AFFINITY

Gain of Function via Strengthening of Interactions

The assembly of proteins into complexes can have several functional benefits, such as increasing protein stability and forming catalytic sites (5, 10, 55, 76). Therefore, mutations that strengthen protein–protein interactions can often cause a gain of function. For example, the S87L mutation in the ATPase MORC2 causes Charcot–Marie–Tooth disease by increasing the stability of the ATP-bound MORC2 dimer (34). MORC2 is an accessory member of the human silencing hub (HUSH), a protein complex capable of silencing genes newly integrated into the genome by chromatin compaction (114). MORC2 binds DNA and then dimerizes via its ATPase module. Here, ATP binding triggers dimerization, while ATP hydrolysis initiates dimer dissociation. The DNA duplexes bound to each MORC2 monomer are brought together upon dimerization, which may promote DNA loop formation and chromatin compaction. The increased dimerization by S87L enhances HUSH silencing, acting via a gain-of-function mechanism. By contrast, severe spinal muscular atrophy has been associated with decreased HUSH silencing via a different MORC2 mutation, T424L (34). This mutation causes a loss of function by increasing ATP hydrolysis and perturbing dimerization dynamics. Although operating via opposite mechanisms, both mutations cause neuropathies. This highlights the utility of considering global reaction equilibriums, rather than isolated changes in a reaction, when investigating sets of disease mutations that appear to operate via different mechanisms.

Mutations can also disrupt biochemical pathways through an increase in binding affinity to small molecules, thereby causing disease via a gain of function. For example, the R233W mutation in cellular retinaldehyde-binding protein (CRALBP) has been associated with retinal pathology. Here, the mutant binds retinoid molecules tighter than the wild type, hindering retinoid dissociation from CRALBP and delivery to the protein retinol dehydrogenase 5, which are essential functions for the visual cycle and oxidation of 11-*cis*-retinol (45, 93).

Ryanodine Receptors: Altered Binding Affinity with Activators Versus Inhibitors

Mutations that increase binding to activating ligands and those that decrease binding to inhibitory ligands can both result in a gain of function. To demonstrate this, we shall consider in more detail the ryanodine receptors (RyRs), which are large, homotetrameric, intracellular ion channels responsible for Ca^{2+} release from the sarco- or endoplasmic reticulum into the cytoplasm, an essential process for muscle contraction (67). Various muscular disorders have been associated with mutations in *RYR1*, which encodes the skeletal muscle isoform, while cardiac arrhythmias such as catecholaminergic polymorphic ventricular tachycardia (CPVT) are caused by mutations in *RYR2*, which encodes the cardiac isoform.

RyR channel opening can be triggered by Ca^{2+}-induced Ca^{2+} release, whereby one of the initial steps requires the binding of Ca^{2+} to an activating binding site (36). It is believed that Ca^{2+} can act as both an agonist and an antagonist for RyRs, depending on whether it binds to a highly selective activating site at low concentrations (A-site) versus a low-affinity inhibitory site at higher concentrations (I-site) (80). Other small molecules are also known to activate the release of Ca^{2+}

(including ATP, caffeine, and nitric oxide) and inactivate RyR (such as magnesium, which acts as a competitive antagonist for the A-site and agonist for the I-site) (36, 64, 85).

Many pathogenic gain-of-function missense mutations that enhance Ca^{2+}-induced Ca^{2+} release and RyR activity have been identified in *RYR1* and *RYR2* via functional studies (38, 81, 131). For example, binding assays of the *RYR1* mutant G2434R demonstrated a decreased binding affinity for both Ca^{2+} and Mg^{2+} agonists of the I-site and enhanced sensitivity for Ca^{2+} over Mg^{2+} for the A-site, which was linked to increased Ca^{2+}-induced Ca^{2+} release activity responsible for malignant hyperthermia (8). Recent advances in cryo-electron microscopy have enabled the identification of putative binding sites at the interdomain interfaces of the C-terminal domain for activating ligands Ca^{2+}, ATP, and caffeine (32) (**Figure 3*a*,*b***). Combined functional and computational analyses on the novel cryo-electron microscopy structures have identified gain-of-function disease mutations that may promote Ca^{2+} binding and loss-of-function mutations that hinder Ca^{2+} binding (25, 60, 85).

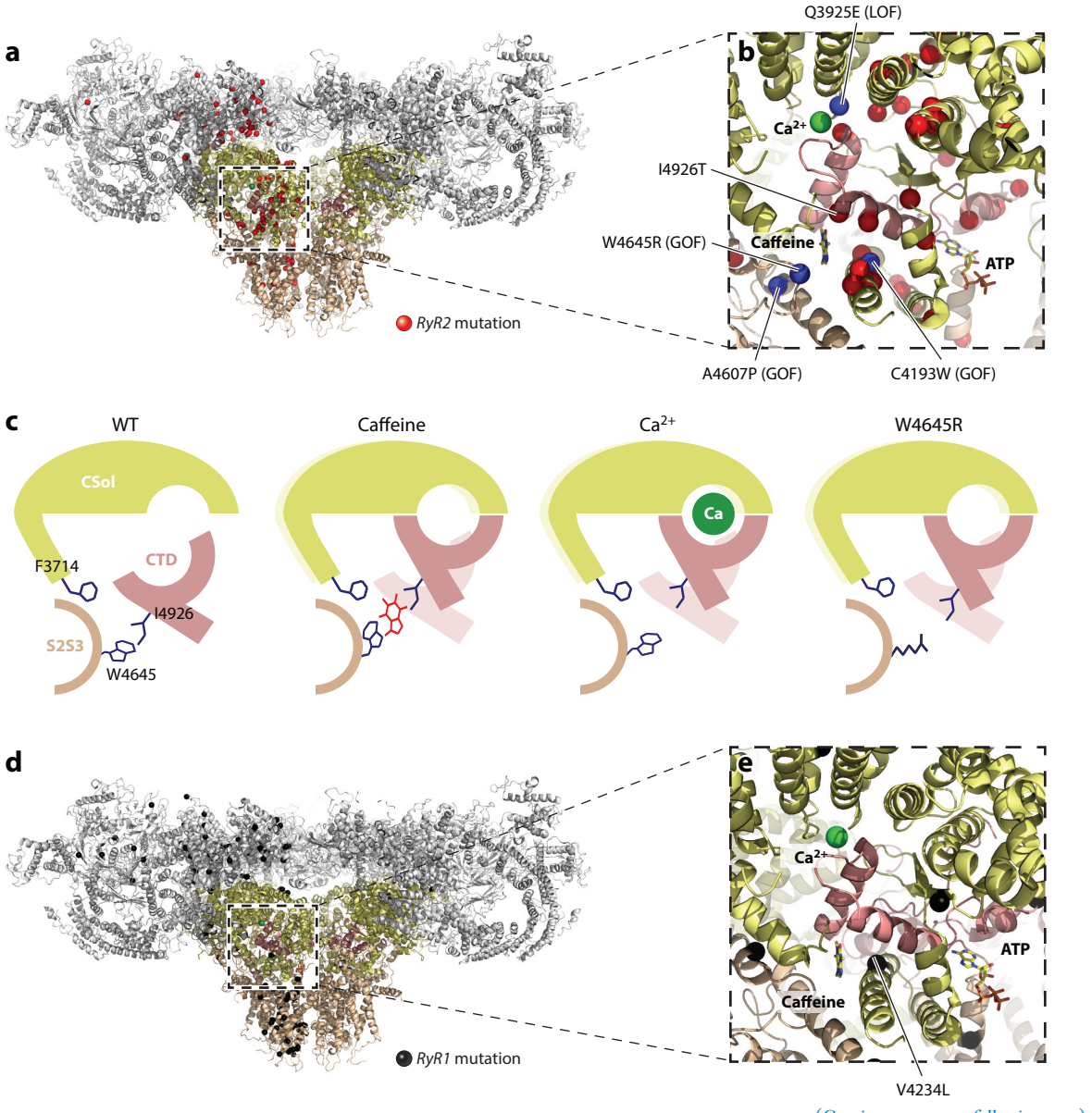
Interestingly, one study of RyR2 identified a tryptophan residue (W4645) in the caffeine-binding site that defines the structure of the Ca^{2+}-binding site to control Ca^{2+} sensitivity (86). In the absence of caffeine, W4645 interacts with an isoleucine residue (I4926), thus enlarging the calcium-binding pocket (**Figure 3*c***). Upon caffeine binding, the interaction is broken, and the calcium-binding pocket is tightened, thus promoting Ca^{2+} binding. The pathogenic W4645R RYR2 mutation is thought to mimic the effects of caffeine binding by breaking the interaction between W4645 and I4926, rendering the calcium-binding pocket tighter and more favorable to calcium binding. In the same study, the authors identified two other CPVT mutations within the caffeine-binding pocket that are capable of enhancing Ca^{2+} sensitivity: C4193W, located just below the C-terminal domain, and A607P, located near W4645 (**Figure 3*b***). Binding studies showed that these mutations increased Ca^{2+} sensitivity eightfold compared with wild-type RYR2 (86). The authors also identified a loss-of-function RYR2 mutation in the calcium-binding pocket (Q3925E) linked to arrhythmogenic diseases that causes reduced Ca^{2+} sensitivity. In fact, many RYR2 disease mutations appear to be clustered within interdomain interfaces of the C-terminal domain, close to these activating ligands (**Figure 3*a*,*b***). These mutations include I4926T, which would presumably disrupt the hydrophobic W4645–I4926 interaction. We predict that further analysis may reveal that many of these mutations also operate through loss- or gain-of-binding mechanisms.

Interestingly, no clustering of pathogenic mutations exists at these ligand-binding sites in RYR1 (**Figure 3*d*,*e***), which is notably more tolerant to loss of function than RYR2. Although enhanced Ca^{2+}-induced Ca^{2+} release has been implicated in RYR1 disease mechanisms, RYR1 predominantly releases Ca^{2+} through a mechanism that is independent of Ca^{2+} influx and is instead triggered by transverse-tubule depolarization (89, 104). RYR1 may therefore be less sensitive to mutations associated with Ca^{2+} binding compared with RYR2, generating an interesting avenue for further study: How do homologs tolerate similar mutations?

GTPases: Dominant-Negative Effects, Gain of Function, and the Role of Intracellular Concentration

Mutations that strengthen interactions can reduce the availability of free ligand for the wild-type protein, thus having a dominant-negative effect. Mutations can also inhibit the dissociation of a ligand indirectly by disrupting an interaction that promotes its release. This indirect inhibition is seen with many GTPase mutations that reduce affinity for guanine nucleotides. GTPases switch between inactive GDP-bound and active GTP-bound conformations. This switching is regulated by guanine nucleotide exchange factors (GEFs), which facilitate the release of GDP and thereby promote GTP binding, and GTPase-activating proteins (GAPs), which inactivate the GTPase by

inducing GTP hydrolysis (105). GTPase mutants with reduced affinity for GTP will thus, in effect, bind more tightly to GEFs, thereby sequestering them and preventing them from activating the wild-type protein, yielding a dominant-negative effect (105). For example, D57N in the Rho GTPase Rac2 has been associated with phagocytic immunodeficiency (6, 50, 126) (**Figure 4**). The diminished ability of the D57N mutant to bind downstream effectors can have numerous biological consequences in hematopoietic cells (1, 50, 100, 126). Notably, the mutant results in impaired superoxide generation and migration of neutrophils (126). Rac1, which is highly homologous to Rac2, rescues some of the endogenous Rac2 functions lost in Rac2 deficiency (50). However, there



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Pathogenic mutations at ligand-binding sites of ryanodine receptors (RyRs). (*a*) Sites of human *RYR2* mutations (*red spheres*) mapped to the structure of the rabbit RyR1 (Protein Data Bank ID 5TV9) (32). (*b*) Close-up view of the boxed area in panel *a*, which contains putative activating binding sites of Ca^{2+}, caffeine, and ATP. Blue spheres highlight sites of pathogenic *RYR2* mutations from Reference 86. LOF and GOF denote mutations that cause a loss or gain in calcium sensitivity, respectively. Red spheres indicate sites associated with pathogenic mutations from ClinVar. (*c*) Conformational changes in human RyR2 Ca^{2+}- and caffeine-binding sites caused by interactions with W4645 and I4926. The wild-type (WT) scheme represents the putative conformation in the absence of physiological ligands. Caffeine binding rotates the tryptophan residue and breaks the isoleucine–tryptophan interaction, which leads to a tighter calcium-binding pocket. A similar conformation likely occurs on Ca^{2+} binding. The pathogenic W4645R *RYR2* mutation may also break the interaction, thereby promoting Ca^{2+} binding. The core solenoid (CSol) domain, S2S3 domain, and C-terminal domain (CTD) are colored in yellow, beige, and pink, respectively. Light colors in caffeine, Ca^{2+}, and W4645R represent locations of the CSol and CTD in the WT state. (*d*) Sites of pathogenic *RYR1* mutations (*black spheres*) from ClinVar mapped to rabbit RyR1 (Protein Data Bank ID 5TV9) (32). (*e*) Close-up view of the boxed area in panel *d*, which contains putative activating binding sites of Ca^{2+}, caffeine, and ATP. Panel *c* adapted from Reference 86 (CC BY 4.0).

is also evidence that the D57N Rac2 mutant sequesters GEFs from other Rac-related GTPases, such as Rac1, which likely contributes to the disease phenotype (50, 100).

The GTPase catalytic cycle is sensitive to changes in the concentration of the reactive species, which can influence the resulting molecular mechanism of a mutation. Such is the case for the D119N HRas mutation associated with cancer (103, 125) (**Figure 4**). Oncogenic Ras mutations are commonly located within the nucleotide-binding pocket and tend to cause an accumulation of active Ras-GTP capable of binding and activating a series of effector molecules, promoting cell growth and survival (116). HRas D119N causes both a loss of binding affinity for the nucleotide-free mutant for GDP and a gain in binding affinity for a GEF, such that the affinity for the GEF increases by a factor of 23,000 relative to the affinity for GDP (28). At certain cellular concentrations, the mutation behaves in a dominant-negative manner, whereby the increased relative affinity for GEF over guanine nucleotides enables the mutation to sequester GEF and prevents

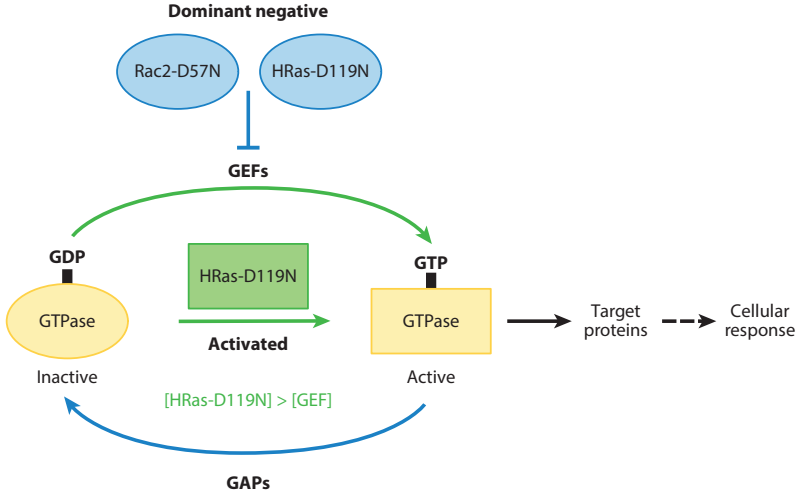


Figure 4

Catalytic cycle of the inactive GDP-bound form to the active GTP-bound form of GTPases. Dominant-negative mutants (Rac2-D57N and HRas-D119N) with reduced guanine nucleotide affinity sequester guanine nucleotide exchange factors (GEFs), preventing them from activating wild-type protein. HRas-D119N is also capable of binding to GTP independent of GEF, thereby behaving as an activated gain-of-function mutant when the concentration of mutant Ras, [HRas-D119N], is in excess of the concentration of GEF, [GEF]. Additional abbreviation: GAP, GTPase-activating protein.

GEF-induced GDP dissociation of the wild-type protein (similar to Rac2 D57N). However, when there is not enough mutant protein to titrate out all of the GEF, the activity of wild-type HRas is not blocked and can still be activated. It has also been proposed that the weakened affinity of the D119N mutant for guanine nucleotides facilitates GDP dissociation, so that GTP can bind independently of GEF. As a result, when the cellular concentration of the mutant surpasses that of GEF, the mutant-GTP complex is activated, behaving more like a gain-of-function oncogenic mutation. Therefore, the molecular mechanism of this mutation and overall phenotype depend on the cellular environment. In this instance, the relative cellular concentration of GTP is 25 times higher than the concentration of GDP, which would support an activated mechanism when the mutant is in excess of GEF (124).

MUTATIONS THAT CHANGE BINDING SPECIFICITY

Some pathogenic mutations act by changing the binding specificity of a protein. While this could be thought of as a combination of weakening certain interactions and strengthening others, the pathogenic mechanisms can often be best understood by considering overall effects on binding specificity.

Toxic Gain of Function via Catalytic Promiscuity

One way this phenomenon manifests is through mutations that increase catalytic promiscuity—i.e., the protein acquires a new substrate specificity or catalytic function. Many enzymes have residual activities for different ligands, and mutations can shift these activities, which has been studied from an evolutionary perspective (117). An instance of catalytic promiscuity causing disease is seen with a peripheral late-onset sensory neuropathy [hereditary sensory and autonomic neuropathy type 1 (HSAN1)], caused by the C113W mutation in *SPTLC1*, which encodes the long-chain base subunit 1 (LCB1) of the protein serine palmitoyltransferase (SPT) (40). Wild-type SPT condenses serine with different-length acyl–coenzyme A (acyl-CoA) substrates depending on the isozyme (52). Two heterodimers exist, which are made of a common LCB1 subunit and one of the two LCB2 subunit isoforms (LCB2a or LCB2b). The addition of a small subunit to form heterotrimeric SPT boosts the catalytic activity (52). While the affinity of the mutant SPT isozymes for serine remains similar to that of the wild type, both the mutant heterodimeric and heterotrimeric SPT complexes are less catalytically active. This, along with the fact that other HSAN1 mutations result in loss of SPT activity (58), suggests that the phenotype could be due to haploinsufficiency. However, haploinsufficiency has been contradicted by the observation that some HSAN1 patients have increased levels of SPT activity, suggesting a gain-of-function mechanism (31). Indeed, the mutant C133W heterotrimeric SPT isozymes have an enhanced ability to condense alanine with their preferred acyl-CoA substrates, yielding 1-deoxysphinganines, which induce an endoplasmic reticulum stress response and behave as a possible neurotoxic agent. Note that the mutant has not acquired an increased affinity for alanine, but instead has an altered active site thought to stabilize reactive intermediates of the condensation reaction of alanine. Further reports of *SPTLC1* mutations S331F and A352V leading to elevated levels of deoxysphingoid bases in HSAN1 patient plasma samples support the theory that the disease phenotype is caused by catalytic promiscuity (101).

Gain of Function via Changes in Ligand-Binding Specificity

Gain-of-function mutations that constitutively activate a protein through altered ligand-binding specificity have also been reported. The human mineralocorticoid receptor (MR) is a transcription factor encoded by the *NR3C2* gene. When activated, it stimulates the transport of sodium,

potassium, and water, which raises blood pressure and extracellular fluid volume (99). Steroids with a 21-hydroxyl group, such as aldosterone and deoxycorticosterone, activate MR by forming a hydrogen bond with residue N770 that stabilizes its active conformation. Steroids lacking the 21-hydroxyl group, such as progesterone, are capable of binding to MR but are unable to form the activating contact at N770, thus behaving as antagonists. Conversely, the mutation S810L in MR establishes multiple van der Waals contacts within its helix-3 that promote the active conformation so that, while ligand binding is required to fully activate MR, formation of the hydrogen bond between N770 and the 21-hydroxyl group is not necessary (13). Furthermore, S810L enhances binding affinity to steroid ligands (98) due to an interaction formed between the mutated leucine and the C-19 methyl group of the steroids (13). Here, MR antagonists become S810L MR agonists. In humans, progesterone tends to be at a higher concentration than MR agonists, particularly during pregnancy (61). As a result of the constitutive activation of S810L MR, individuals who carry this mutation experience early-onset hypertension, which is heightened during pregnancy. Here, both increased ligand binding and loss of binding specificity contribute to MR deregulation through nonspecific binding to the large number of steroid molecules present in the body.

MUTATIONS THAT RESULT IN ASSEMBLY-MEDIATED DOMINANT-NEGATIVE AND DOMINANT-POSITIVE EFFECTS

Dominant-Negative Poisoning of Protein Complexes

While dominant-negative effects can sometimes occur via competition-based mechanisms, in which there is no direct interaction between the wild-type and mutant proteins, the majority of known dominant-negative mutations are dependent on the mutant protein being able to coassemble into a complex with wild-type subunits. If the mutant protein can poison the activity of the hybrid wild-type:mutant complex, it will cause a disproportionate loss of function, as most of the complexes that assemble will contain at least one mutant subunit (9, 121). For example, in the case of a heterozygous mutation in a gene encoding a homotrimer, seven of eight complexes will contain a mutant subunit (**Figure 5**), assuming that assembly is not occurring cotranslationally (90).

Mechanistically, there are several ways that the presence of a mutant subunit within a hybrid wild-type:mutant complex can be damaging. Often, the hybrid complex is simply nonfunctional, as is the case for ferritin light chain (FTL), where dominant-negative mutants associated with neurodegeneration can assemble with wild-type subunits into an octahedral 24-mer complex, but with a greatly reduced ability to incorporate iron compared with the fully wild-type complex (74). Alternatively, the incorporation of a mutant subunit can exert a dominant-negative effect by causing mislocalization of the complex, as seen for the homotetrameric HCN4 channel, where the dominant-negative D553N mutation associated with cardiac arrhythmia interferes with trafficking of hybrid complexes to the plasma membrane (118). The coassembly of mutant and wild-type subunits can also cause enhanced degradation of both subunits, a process that has been called procollagen suicide in the case where mutant procollagen α-subunits promote the degradation of the wild-type subunits with which they interact (96).

Although there are many examples of human genetic disorders caused by assembly-mediated dominant-negative effects, the precise molecular details of how the mutant proteins are able to disrupt wild-type activity are rarely known. In part, this is because it would be extremely difficult to experimentally characterize the structure of a hybrid wild-type:mutant complex, due to the inherent heterogeneity of assembly, and so precise details of the interactions between mutant and wild-type subunits remain elusive. In some cases, however, it has been possible to solve the structures of dominant-negative mutants in a homogeneous state (26, 68). For example, a crystal

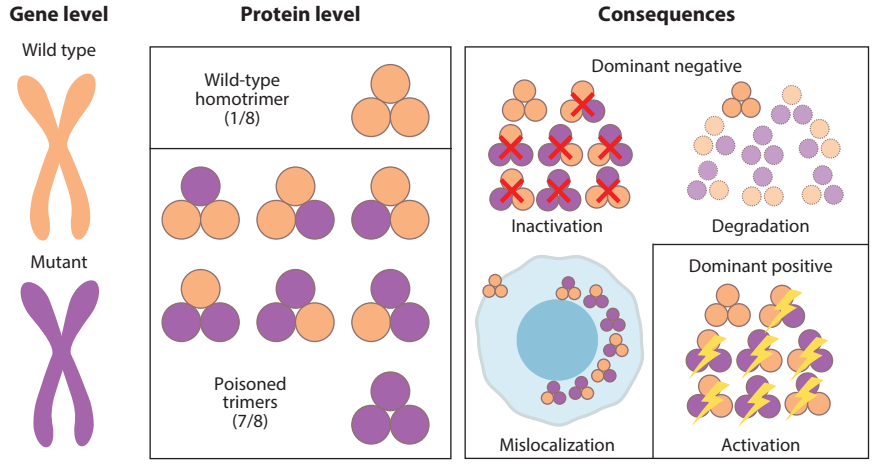


Figure 5

Assembly-mediated dominant-negative and dominant-positive effects. For a hypothetical homotrimeric protein complex, where wild-type and mutant subunits are expressed at equal levels, seven of the eight possible trimers that form will contain at least one mutant subunit. The presence of a mutant subunit in a trimer can poison its activity and cause a dominant-negative effect in various ways, including inactivation, mislocalization, and degradation. A mutant subunit can also cause increased activation, leading to a dominant-positive effect.

structure was determined for one of the dominant-negative FTL mutations mentioned above, which introduces a 16-residue insertion at the C terminus. This mutant can assemble into a ferritin shell with an efficiency comparable to the wild type's. However, analysis of the mutant crystal structure reveals a loss of stabilizing interactions around the intersubunit interface and the formation of a large opening in place of the tight hydrophobic channel, thus accounting for the dramatic reduction in the ability of the wild-type:mutant complex to incorporate iron (74).

Given that most examples of the dominant-negative effect rely on the mutant protein still being able to assemble into a complex, it is not surprising that dominant-negative missense mutations have been observed to be much milder at a protein structural level than loss-of-function mutations, with much smaller predicted destabilizing effects (43, 77). In general, highly disruptive mutations should not be compatible with a dominant-negative effect. There are exceptions, however, as was observed for mutations associated with late-onset retinal degeneration in *C1QTNF5*, where the structurally damaging effects of dominant-negative mutations could be rationalized by the fact that the protein assembles via two distinct regions (14, 107). Specifically, although the pathogenic mutations are highly destabilizing to the trimeric gC1Q domain, this does not preclude higher-order oligomerization into an 18-mer via the collagen domain.

Most examples of dominant-negative mutations involve homomeric protein complexes, composed of multiple copies of the same polypeptide chain, or heteromeric complexes that contain homomeric interactions between repeated subunits [e.g., microtubules, made up of repeated copies of α- and β-tubulin subunits (3)]. This provides a way for the mutant to affect the wild-type protein, by assembling together within the same complex. However, there have been reports of dominant-negative mutations affecting heteromeric complexes that have no repeated subunits. For example, a reported dominant-negative mutation (G41R) causing renal hypomagnesemia was identified in the γ-subunit of the heterotrimeric sodium-potassium ATPase, encoded by *FXYD2* (79). Incorporation of the mutant subunit leads to misrouting of the entire complex away from the plasma membrane. In this case, while the other subunits of the complex are affected, there is no direct

impact on the wild-type γ-subunit. Whether the mutation is truly dominant negative depends, in effect, on the intracellular concentrations of the other ATPase subunits. If they are produced in excess and all of the wild-type γ-subunit is able to assemble, then a heterozygous loss-of-function mutation should show an equivalent phenotypic effect. However, if the mutant competes with the wild-type protein for binding to the other subunits, then it would be best described as a competitive dominant-negative effect. Given that individuals with heterozygous *FXYD2* deletions have normal serum Mg^{2+}, G41R is likely a true dominant-negative mutation.

Dominant-Negative Versus Dominant-Positive Effects

The phenomenon of mutant subunits poisoning the activity of a protein complex is often simply referred to as the dominant-negative effect. However, as we have seen, dominant-negative effects can also occur in other ways, such as via competition. Furthermore, the formation of a hybrid wild-type:mutant complex can sometimes lead to a gain of function. This has been referred to as a dominant-positive or dominant-activating effect, in analogy to the dominant-negative effect. We suggest using the terminology assembly-mediated dominant-negative and dominant-positive effects when discussing these phenomena to distinguish them from other dominant-negative and gain-of-function mechanisms and to emphasize the fundamental mechanistic similarity between these processes. **Figure 5** illustrates different ways in which assembly-mediated dominant-negative and dominant-positive effects can occur.

One example of an assembly-mediated dominant-positive effect involves the homodimeric TASK-4 potassium channel, encoded by *KCNK17*, where the mutation G88R has been associated with arrhythmia. This mutation, which occurs in an extracellular loop region, causes a clear gain of function by increasing channel activity (39). Moreover, coexpression studies suggested that the mutant could also hyperactivate the hybrid channel formed upon interaction with the wild-type subunit, thus leading to an enhanced gain of function. In effect, this is the opposite of the disproportionate loss of function observed with dominant-negative mutations, driven via a similar molecular mechanism.

MUTATIONS THAT CREATE NOVEL INTERACTIONS

While mutations that change binding specificity will tend to alter the relative affinities for different natural ligands, or cause binding to ligands that are closely related via the same interface, it is possible for mutations to create genuinely novel interactions. While it may seem unlikely for a single mutation to result in a new interaction, on average, it takes only two amino acid substitutions to convert a patch on the protein surface to the composition of a typical interface (72). Furthermore, protein design experiments have shown that a single mutation can be sufficient to induce higher-order self-assembly (49). Thus, it is reasonable to expect that individual missense mutations might occasionally result in new binding interfaces. However, it is likely that such mutations will be underrepresented in the literature compared with their true occurrence because, while it is usually simple to show that a mutation is disrupting a known function, it is less likely that researchers will perform the correct experiments to discover a novel, mutation-specific interaction.

Protein Mislocalization Due to Gain of Dileucine Motif

Many protein interactions and posttranslational modifications occur via short linear motifs (SLiMs), which are typically less than 10 residues long and located within intrinsically disordered regions (115). The importance of SLiMs is reflected in the fact that mutations that disrupt them can be pathogenic (119). Research has shown that intrinsically disordered regions have a higher

capacity to rewire the interactome on an evolutionary timescale compared with structured domains (84). Thus, from a purely statistical view, it could be considered more probable for a single mutation in a disordered region to form a novel interaction via the gain of a SLiM than for a single mutation within a structured domain to do so.

Three missense mutations in disordered cytosolic regions of different transmembrane proteins (GLUT1, ITPR1, and CACNA1H) were found to cause neurological diseases (82). All three involve proline-to-leucine substitutions and result in the creation of a dileucine motif—a SLiM that is known to recruit clathrin and mediates endocytosis and intracellular trafficking of plasma membranes (92). Further investigation of the GLUT1 mutant revealed that clathrin binding enables the recruitment of several vesicular transport adaptor proteins, which causes mislocalization of the GLUT1 receptor from the plasma membrane to intracellular compartments. This mislocalization prevents the receptor from mediating glucose transport in cells, which in patients with the heterozygous GLUT1 mutation causes GLUT1 deficiency syndrome 1 (69, 82). In this case, although the mutation results in a new interaction via the gain of a SLiM, the overall disease mechanism is a loss of function due to protein mislocalization.

Gain of Posttranslational Modification Sites

Given that posttranslational modifications often occur at simple motifs such as SLiMs, it is relatively simple for mutations to create new posttranslational modification sites. This can be thought of as gaining a new interaction between the protein and the modifying enzyme. For example, T168N in *IFNGR2*, which encodes the IFNγR2 chain of the IFNγ receptor, is associated with a rare autosomal recessive immunodeficiency, where patients are prone to mycobacterial infections (122). The IFNγ receptor is a tetramer made up of two IFNγR2 chains and two ligand-binding IFNγR1 chains, which forms upon IFNγR1 binding to the cytokine IFNγ. This mutation introduces an *N*-glycosylation consensus sequence that leads to the addition of a novel *N*-linked glycan, which abolishes the cellular response to IFNγ. Exactly how the additional carbohydrate moiety blocks the IFNγ response remains unclear. It has been suggested that the *N*-glycan may sterically hinder IFNγ–IFNγR1 or IFNγR1–IFNγR2 interactions, which would block the IFNγ-induced tetramerization, or that it could inhibit the function of the assembled tetramer, for instance, by disrupting the folding of the complex or its subcellular localization (123). Additionally, four *IFNGR2* hypomorphic mutations (R114C, S124F, G141R, and G227R) associated with partial IFNγR2 immunodeficiency result in misfolded IFNγR2 proteins with abnormal *N*-glycosylation that are mostly retained in the endoplasmic reticulum (83). In fact, a loss of function through the mutational gain of a glycosylation site may be implicated in a variety of disorders, as missense mutations predicted to create a novel glycosylation site are significantly enriched in disease genes encoding proteins trafficked through the secretory pathway (122). Encouragingly, *N*-glycosylation inhibitors improved the IFNγ response of patients' cells with the hypomorphic and complete loss-of-function *IFNGR2* mutations (83, 122). Therefore, further characterization of gain-of-glycosylation mechanisms may have wide clinical benefits by raising the possibility of targeted treatment with glycosylation inhibitors.

Another example of a pathogenic gain of posttranslational modification is the introduction of a phosphorylation site by the H878Y mutation in HER2, encoding the receptor tyrosine-protein kinase ErbB2, which is associated with hepatocellular carcinoma (59). When activated, ErbB2 autophosphorylates its C-terminal tyrosine residues, thus triggering a network of signaling pathways (133). Constitutive activation of ErbB2 has been linked to the tumorigenesis of various cancers. The phosphorylated Y878 residue, which is located in the activation loop of the kinase, forms a salt bridge interaction with R989 and stabilizes the active conformation of the mutant, thereby

increasing its kinase activity. In this instance, the gain of a posttranslational modification site leads to an overall gain of function by forming a constitutively activated protein.

Gain of New Protein–Protein Interactions

Although less frequently reported, some mutations within structured domains can lead to new protein–protein interactions. Sometimes these new interactions can yield toxic effects, which can cause disease. For example, the G406R mutation in *CACNA1C*, which encodes a voltage-dependent calcium ion channel (CaV1.2), creates a novel binding site for calcium/calmodulin-dependent protein kinase type II (CaMKII) (37). This mutation causes a type of long QT syndrome called Timothy syndrome that affects the heart, the brain, and several other organs. CaV1 channels conduct excitation coupling by mediating the influx of calcium ions into the cytoplasm, which plays a particularly important role in heart contractility and neuronal development. Experiments showed that this mutation-induced interaction triggers the CaMKII-dependent phosphorylation of CaV1.2 at nearby residue S409. Phosphorylation at this site causes longer channel openings and leads to cytotoxic levels of calcium in the cytoplasm.

Mutations that create protein–protein interactions have also been implicated in several cancers. The *PIK3CA* gene encodes the catalytic subunit (p110α) of phosphatidylinositol 3-kinase alpha (PI3Kα), whose activity is inhibited by the regulatory subunit (p85) of PI3Kα in the basal state (120). When stimulated by growth factors, p85 binds insulin receptor substrate 1 (IRS1), which activates PI3Kα and promotes cell proliferation and survival by activating the AKT pathway (19). A somatic mutation (E545K) in the p110α domain, frequently observed in human cancers, gains the ability to interact with IRS1 directly (53), which allows the oncogenic AKT pathway to be constitutively activated independent of growth factors.

The cancer-associated R273H mutation of the tumor suppressor p53 gains a novel interaction with nardilysin that promotes cellular invasion toward the heparin-binding EGF-like growth factor (27). Here, the mutant gains the ability to promote invasion via a novel protein–protein interaction. In fact, R273H preferentially interacts with several novel binding partners involved in processes such as cell division, polarity, and adhesion (27).

MUTATIONS THAT PROMOTE PROTEIN AGGREGATION AND PHASE SEPARATION

A number of human genetic disorders are caused by mutations that induce protein aggregation, the process by which misfolded proteins self-assemble into large aggregates. These aggregates often take the form of highly organized structures known as amyloid fibrils, which are rich in cross-β secondary structure (65). If the wild-type protein does not aggregate, then we can consider these aggregation-inducing mutations to be gaining new self-interactions. By contrast, if the wild-type protein can already aggregate in the absence of mutant protein, then these aggregation-inducing mutations may be considered a form of strengthened interactions.

There are multiple mechanisms by which an aggregation-inducing mutation could cause disease. Aggregation may simply cause a loss of function, analogous to protein destabilization, as the aggregated protein is no longer able to perform its normal biological role. Similarly, if the mutant protein can also induce the wild-type protein to aggregate, this could cause a disproportionate loss of function and thus have a dominant-negative effect. However, most research suggests that the primary way in which aggregation causes disease is via toxic gain-of-function effects. In some cases, it is not the aggregates themselves that are damaging, but the much smaller, soluble protofibrils that form earlier in the aggregation process. These protofibrils may have a variety of damaging

effects, such as perturbing and disrupting membranes (11). In addition, protein aggregates and protofibrils may lead to a general impairment in proteostasis (i.e., protein homeostasis), leading to chronic cellular stress and disease progression (57).

At least six different aggregation-inducing missense mutations have been identified in *SNCA*, which encodes α-synuclein, that cause familial Parkinson's disease or Lewy body dementia (17). Recently, the cryo-electron microscopy structures of amyloid fibrils formed by four of these mutants have been determined, providing considerable insight into their molecular disease mechanisms. While wild-type α-synuclein can form fibrils under certain conditions, these mutants all greatly enhance aggregation and involve radical changes in the interfaces formed between different protofilaments (i.e., fibril subunits). For example, both the H50Q and A53T mutations appear to disrupt the interface formed between protofilaments observed in the wild-type fibrils, from residues 50 to 57, and lead to a new, smaller protofilament interface between residues 58 and 61, which may promote fibril dissociation and propagation (15, 111). G51D also disrupts the wild-type interface, instead forming a new protofilament interface involving residues 74–79 (112). By contrast, E46K leads to a substantial conformational shift in the protofilament structure, facilitating a new interface that overlaps the wild-type interface, spanning residues 45–57, and leading to increased fibril stability (16). Thus, there appear to be remarkably different ways in which pathogenic mutations can disrupt and create interactions between protofilaments to promote toxic fibrillation.

The phenomenon of liquid–liquid phase separation (LLPS) is similar to aggregation, in that it involves the indefinite self-assembly of proteins into large condensates, although LLPS has the crucial difference of being much more easily reversible. This self-assembly is typically mediated by multivalent interactions via small interacting domains or intrinsically disordered regions. While there has been much focus in recent years on the functional benefits of LLPS, it is also possible for mutations to cause disease by inducing the formation of condensates not observed in the wild-type protein. For example, mutations in the nonreceptor protein tyrosine phosphatase SHP2, encoded by *PTPN11*, have been associated with developmental disorders. Activating mutations that increase phosphatase activity can cause Noonan syndrome, while inactivating mutations can cause a related disorder, Noonan syndrome with multiple lentigines (66). A study recently demonstrated that both types of pathogenic SHP2 missense mutations gain the ability to undergo LLPS (136). At a molecular level, these mutants shift the protein to a more open conformation that exposes charged patches on the protein surface, which can then participate in the electrostatic interactions that drive LLPS. Crucially, the wild-type protein also coassembled with the mutants into the condensates. Moreover, the formation of condensates enhances phosphatase activity, likely due to increased local concentration (4). Taken together, these findings can explain the phenotypic similarities between activating and inactivating SHP2 mutants: Given that they both drive the formation of LLPS condensates, which also contain wild-type proteins, and that LLPS enhances phosphatase activity, even the inactivating mutations lead to a gain of function, due to the increased activity of the wild-type protein. Mechanistically, this process resembles the assembly-mediated dominant-positive effect discussed above, the key difference being that the wild-type protein does not normally self-assemble by itself.

OUTLOOKS AND CHALLENGES

We have highlighted diverse molecular mechanisms by which protein mutations can cause human genetic disease. One reason why it is so important to understand molecular mechanisms is because it can lead to improved identification of pathogenic mutations and thus diagnosis. Non-loss-of-function mutations are more poorly predicted by current computational methods than

loss-of-function mutations (43). In the future, knowledge and understanding of molecular mechanisms may be used to improve computational predictions. For now, however, researchers should be aware that gain-of-function and dominant-negative mutations are more likely to be missed if strict filtering based upon the scores of computational predictors is used. This is especially important when considering dominant disorders and when searching for de novo variants, where non-loss-of-function mechanisms will be far more prevalent. Researchers should consider paying more attention to variants that may not meet the strict pathogenicity thresholds used by computational predictors, but where gain-of-function or dominant-negative mechanisms could be plausible.

One scenario where consideration of alternative molecular mechanisms may be particularly useful is when heterozygous missense variants are identified in a gene that has typically been associated with a recessive disorder. It may be tempting to exclude such variants as disease candidates, under the assumption that they would not be pathogenic in a heterozygous state. However, as the dominant-negative effect leads to a disproportionate loss of function, we occasionally see cases where heterozygous dominant-negative mutations cause the same disorder as biallelic loss-of-function mutations in the same gene. For example, both dominant-negative (77) and homozygous loss-of-function (44) mutations in *ITPR1*, which encodes a homotetrameric inositol triphosphate receptor, cause Gillespie syndrome. If a protein is known to form a homomeric complex, then a dominant-negative effect from heterozygous variants could be plausible and should be investigated in more detail.

Considering alternative molecular mechanisms is also important for understanding cases where different mutations in the same gene are associated with different clinical phenotypes. For example, destabilizing missense mutations in the PAX6 transcription factor cause haploinsufficiency and are associated with classical aniridia (an anterior segment eye disorder), whereas gain-of-function mutations that perturb DNA binding can cause more severe phenotypes, such as microphthalmia (127). Thus, awareness that different phenotypes may be associated with mutations that act via different molecular mechanisms could aid in the identification of novel pathogenic mutations.

Although understanding the molecular mechanisms underlying disease mutations is important, it is sometimes very difficult. Experimentally, it is often simple to show that a mutation is destabilizing, and computational methods are fairly good at using protein structures to predict pathogenic destabilizing mutations (42). However, since the effects of many disease mutations are related to intermolecular interactions, we can explain their mechanisms only if we understand the relevant interactions. In many cases, we may not even know an interaction exists, let alone have a three-dimensional structure of it. Therefore, it is important to keep in mind that more complex, non-loss-of-function molecular disease mechanisms are almost certainly underreported in the literature.

A further complication is the phenomenon of allostery, whereby mutations that affect interactions may not necessarily involve residues that are directly in the protein interface (94). Disease-causing mutations are often located at residues connected to the binding site via hydrogen bond networks, as is the case with the R1380L SUR1 mutation discussed above (30). In fact, small-molecule binding and catalytic activity can be modified by mutations remote from the active site—for instance, over 12 Å away in the case of copper nitrate reductase (70), or even 20 Å for TEM-lactamase (2). Thus, even having the structure of a protein in complex with its relevant interaction partner may not be enough to understand the effects of a mutation.

In this review, we have focused on molecular mechanisms related to protein interactions, be they with other proteins, nucleic acids, or small molecules. Given that intermolecular interactions are fundamental to essentially every biological process, it is difficult to imagine any pathogenic, non-loss-of-function mutations whose phenotypic effects cannot be understood through their influence on interactions. For example, mutations that enhance catalytic activity can be explained

by their effects on substrate interactions, as we have discussed. Mutations that increase protein stability could possibly have gain-of-function effects, but even this is likely to be mediated by interactions. For example, while gain-of-function missense mutations associated with IMAGe (intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital abnormalities) syndrome increase the stability of CDKN1C, this was caused by disruption of interactions with the proteasome (51).

Finally, understanding molecular mechanisms can provide a pathway toward treatment. For example, better understanding of mutations at small-molecule binding sites can aid drug discovery. The related phenotypic effects of mutations in different genes may be explained through knowledge of molecular mechanisms and effects on downstream pathways. While gene replacement therapy holds great potential for disorders associated with loss-of-function mutations, it is unlikely to work for gain-of-function and dominant-negative mutations, where the mutated alleles would instead need to be edited (20). By contrast, non-loss-of-function mutations will tend to involve much milder perturbations of protein structure, which means they may be far more amenable to treatment via small molecules (23). For example, in patients with gain-of-function mutations causing KCNA2 encephalopathy, the potassium channel blocker 4-aminopyridine was recently shown to be highly effective in reducing symptoms (54). Thus, knowledge of molecular mechanism can be crucial for deciding on a therapeutic strategy.

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Errata

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