Cytotoxicity of Aberrant Proteins

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Article Contents

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A wide range of diseases are caused by the generation of aberrant proteins that for one reason or another are not functionally equivalent to the normal version. Here, we consider the basic cellular principles that govern the generation of aberrant proteins, their normal metabolism and in the case of disease, their adverse effects on cellular function.

Overview: Proteins and Cellular Organization

A typical mammalian cell has approximately10^9–10^{10} individual protein molecules represented by around 10^4–10^5 distinct proteins. In order for this enormous ensemble of proteins to coordinately carry out the biochemical processes critical to the life of a cell, they must be highly organized. Cellular organization spans multiple levels (**Figure 1**) including structural, spatial, temporal and quantitative dimensions that collectively define a cell's morphology and physiology. Indeed, most differences between diverse cell types such as a neuron and hepatocyte are largely due to both the ensemble and organization of their constituent proteins. Hence, there is an intimate relationship between protein organization and cellular function. Conversely, cellular dysfunction is often the result of *aberrant proteins* falling outside a cell's normal organizational scheme. Thus, proteins that are structurally flawed, mislocalized, expressed at the wrong time, or present in the wrong amounts are all defined as aberrant and can impact cellular function in many ways that range from inconsequential to catastrophic.

Remarkably, aberrant proteins arise all the time by any of numerous mechanisms: intrinsic limitations in the fidelity of biosynthetic, trafficking and degradation pathways; extrinsic influences such as changes in temperature, pH, oxidation or salinity; exposure to protein-damaging agents; or in rare circumstances, inherited genetic mutations. To deal with this constant barrage, cells have evolved numerous 'quality control' (QC) mechanisms to recognize and destroy aberrant proteins. Nonetheless, constant aberrant protein production in many conditions, especially over long time periods, can lead to disruptions of normal cellular homeostasis that eventually contribute to disease. The various mechanisms by which aberrant proteins lead to cellular dysfunction and disease will be the topic of this article.

Rather than cataloging a series of incompletely understood and disparate diseases caused by aberrant proteins, we will focus on general principles. By considering the *normal pathways* of protein biosynthesis, trafficking and metabolism (Part I), we hope to illustrate how and why deviations from the norm lead to aberrant protein production, and the mechanisms by which they can cause cellular dysfunction (Part II). Each of these basic cell biological principles will be highlighted by specific examples taken from diseases that are the direct consequence of aberrant proteins generated from mutated genes. No single disease can best exemplify all of the mechanisms, and no unifying mechanism is likely to fully explain any one disease, much less all diseases. Instead, by understanding the general framework for the *possible* ways in which aberrant proteins can cause cellular dysfunction, the concepts outlined here can be used to formulate rational models of pathogenesis for individual diseases on a case-by-case basis.

Part I: The Life of a Protein from Birth to Death

Before considering how aberrant proteins can be harmful, it is productive to clearly delineate the *normal* pathways of protein biosynthesis, trafficking, function and degradation. It is after all a deviation from these pathways that not only generates aberrant proteins, but leads to the downstream consequences. Thus, in the following two subsections, the normal events in the biosynthesis and metabolism of proteins are described, followed by the normal pathways

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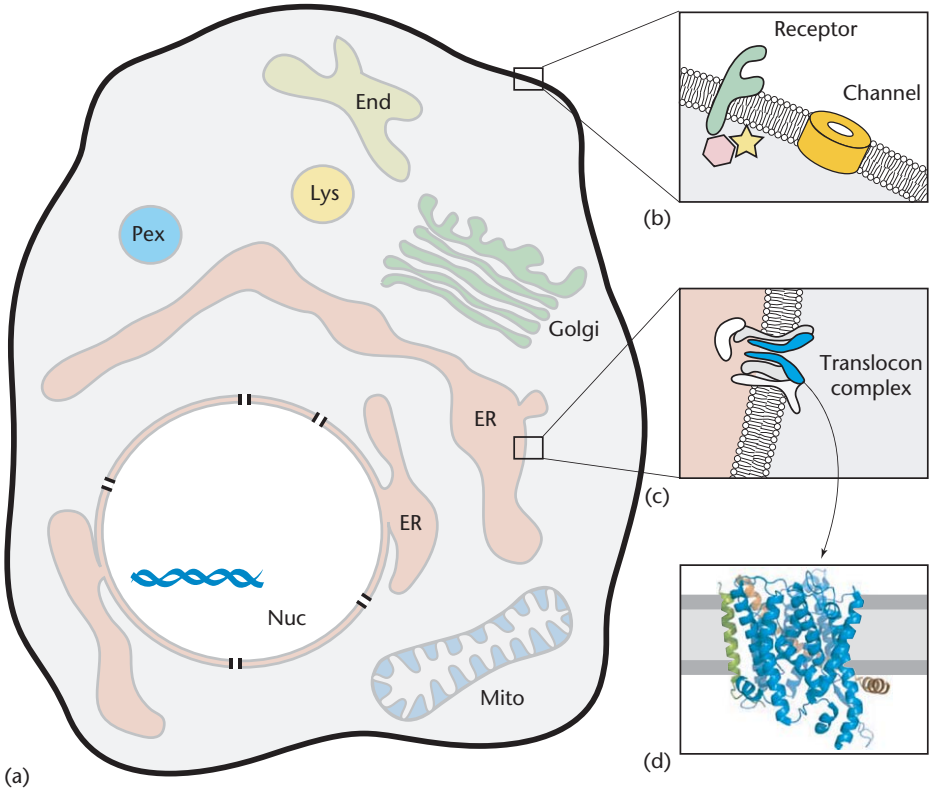

Figure 1 Cellular organization of proteins. An illustration of the multiple layers of cellular organization that must be constantly maintained by biosynthetic and trafficking pathways, and vigilantly monitored by quality control pathways. (a) Eukaryotic cells contain numerous *spatially* distinct compartments whose environments differ considerably. For example, the endoplasmic reticulum (red) is an oxidizing environment, the cytosol is a reducing environment, the cell surface (yellow) is exposed to the outside, endocytic and lysosomal compartments (green) are acidic, and so on. (b, c) At the molecular level, each compartment is filled with its own unique ensemble of proteins (and other macromolecules) whose precise *composition and levels* directly impact that compartment's function. For example, the cell surface contains channels and receptors whose identities and amounts directly impact communication between the inside and outside of the cell, while the ER contains machinery for protein translocation (the 'translocon'). Molecular level organization is also determined by the assembly of many protein constituents into appropriate *functional complexes*. The ER translocon is depicted as an assembly of a channel component (dark grey) associated with various additional factors in the lumen and membrane. The receptor associates with cytosolic signalling molecules to function. (d) At the atomic level, each protein acquires a precise three-dimensional *folded conformation* that allows it to function. Shown is the structure of the channel-forming component of the ER translocon (a heterotrimeric protein called the Sec61 complex).

of quality control that deal with the inevitable generation of aberrant proteins associated with intrinsic inefficiencies in protein biosynthesis.

Protein biosynthesis, trafficking and metabolism

Newly synthesized polypeptides must fold into their native 3-dimensional conformations, acquire any number of post-translational modifications, and often assemble with other proteins to achieve a correctly functional product (Dobson, 2003). During these synthesis and maturation steps, a large range of factors, generally termed chaperones, transiently interact with the folding polypeptide to protect intermediate states from inappropriate interactions in the highly crowded environment of a cell. In other instances, chaperones catalyze specific reactions, such as disulfide bond formation or prolyl isomerazation, necessary for some substrates to fold properly. And yet other maturation factors act enzymatically to contribute post-translational modifications such as glycosylation, methylation, proteolytic processing, phosphorylation and numerous others. In this manner, a series of substrate interactions with both chaperones and maturation factors facilitate folding and assembly until the native, biologically active protein is produced. Unless the site of synthesis also happens to be the intended site of function, most proteins must be trafficked to other parts of the cell (or outside the cell in the case of secreted molecules) after their initial maturation. **See also**: Protein Folding and Chaperones

Trafficking of proteins to their final destination is an acutely coordinated process that typically involves the recognition of sorting signals on the newly synthesized protein by a very wide range of specific sorting and transport factors (Wickner and Schekman, 2005; Bonifacino and Traub, 2003; Mellman and Warren, 2000; Rothman and Wieland, 1996). In some cases, multiple signals are employed sequentially. For instance, a transmembrane protein destined for the plasma membrane would contain a signal for first directing it into the ER, followed by a signal for ER exit, and

finally for sorting at the Golgi into vesicles destined for the cell surface. This protein may further mature en route, for example, by the addition or modification of glycans in the Golgi. By contrast, proteins resident to the ER would have a comparable signal for ER entry, but lack sequences for further trafficking; instead, retention signals on the protein would be recognized by yet other factors that actively prevent exit from the ER or retrieve any copies that inadvertently escape to other organelles such as the Golgi. Comparable signals and transport machinery exist for all cellular locations, even those not delimited by a membrane border (e.g. the nucleolus, subregions of the cytosol, or certain parts of a membrane surface). Thus, by employing these systems, a cell has exquisite control over the location of its constituent proteins so that each can perform its intended function appropriately. **See also**: Intracellular Transport

The final facet of a protein's normal life is its degradation (Goldberg, 2003). Not only are all proteins constitutively replenished, but individual proteins that are no longer required are selectively degraded. Protein turnover rates range from minutes to several days, depending on the identity of the protein. For instance, proteins that are important regulators of cellular pathways tend to be degraded more rapidly, as this allows the cell to adapt more effectively to changes in its environment. By contrast, proteins that have scaffolding, structural or housekeeping roles tend to be long-lived. Most protein degradation culminates at one of two cellular components: lysosomes and proteasomes. Lysosomes are highly acidic membrane-bound organelles that contain numerous proteases, glycosidases, nucleases, lipases and other digestive enzymes. With few exceptions, cargos destined for lysosomal degradation are delivered via vesicles that must fuse with the lysosome to access the degradative enzymes. By contrast, the proteasome is a cytosolic multiprotein degradation factor shaped like a barrel that contains several highly regulated proteases on its inner surface. Substrates for proteasomal degradation are covalently tagged with a small protein called ubiquitin by a series of steps involving ubiquitin ligases (Hershko and Ciechanover, 1998). Polyubiquitinated proteins are then degraded to peptides by the proteasome. This highly regulated pathway of cytosolic protein degradation is collectively referred to as the ubiquitin-proteasome system. In addition to these two major pathways, other more specialized pathways exist for degradation in certain organelles (e.g. the mitochondria). **See also**: Lysosomal Degradation of Proteins; Protein Misfolding and Degradation in Genetic Disease

Although the actual mechanisms by which cellular factors select a protein for degradation are poorly understood, the choice of degradative pathway is generally dictated by the cellular locale in which the protein normally resides. Proteins in most membrane-bound compartments (except the ER) are targeted to the lysosome by vesicular transport. Even bulk cytosol or whole organelles can be routed to lysosomes through a process called autophagy (Klionsky, 2007). Most cytosolic, nuclear and ER proteins utilize the proteasomal degradation machinery. In the case of ER proteins, they must be first extracted from the membrane (or retrotranslocated from the lumen) into the cytosol before degradation (Meusser *et al*., 2005). Because degradation is both spatially and temporally regulated, the amounts of specific proteins at particular cellular locations are substantially influenced by its degradation, making this facet of a protein's life every bit as important to its function as its biosynthesis and trafficking. Indeed, the functional expression of certain proteins is controlled almost entirely by regulation of their rate of degradation.

Protein quality control and degradation of aberrant proteins

As described earlier, numerous cellular pathways are dedicated entirely to synthesizing a protein in the right amount (balance between biosynthesis and metabolism), ensuring its proper folding and maturation (chaperone interactions) and delivering it to the right place (trafficking) to carry out its intended function. Despite this, intrinsic inefficiencies in each process can lead to the generation of aberrant proteins even under the most optimal conditions. The cell has therefore evolved multiple *quality control* (QC) mechanisms that are designed to recognize and eliminate aberrant proteins (Ellgaard and Helenius, 2003; McClellan *et al*., 2005). The quality control systems that act at the steps of protein maturation are best understood. **See also**: Quality Control of Protein Folding in the Cytosol

Chaperone–protein interactions, essential for protein maturation, are also a central component of most cellular QC systems. It is thought that prolonged interaction of a protein with chaperones is a key step in selecting the protein for degradation. This is logical because chaperones typically interact with non-native proteins that are not folded correctly. Although the precise features that chaperones recognize as 'non-native' are not defined completely, one feature seems to be the surface exposure of hydrophobic regions that would be buried in a properly folded protein. Thus, chaperone binding tends to keep a protein from aggregation, presumably by shielding the exposed hydrophobic regions from inappropriate interactions. These properties of chaperone–substrate interactions not only facilitate folding (by preventing inappropriate interactions), but also maintain the protein in a degradation-competent (i.e. unaggregated) state in case folding is unsuccessful. By coupling the triage decision regarding degradation to the timing of chaperone–substrate interactions, a QC mechanism is initiated whereby substrates are afforded an initial opportunity to fold into a native form, after which they are routed into a degradation pathway. One of the best-studied examples of this timer-based system of triage between maturation and degradation is the lectin chaperones (Calenxin and Calreticulin) in the ER.

The mechanisms by which chaperone–substrate complexes are coupled to the degradation machinery during QC is not fully understood and varies considerably

depending on the cellular compartment, the chaperone system involved and the specific degradation pathway. For example, proteins in the ER lumen must be targeted to a retrotranslocation channel through which they must be transported to the cytosol for subsequent degradation. The identity of this channel and the mechanism of targeting are unknown at present; however, once exposed to the cytosol, substrates become polyubiquitinated in preparation for degradation by the proteasome. Cytosolic proteins can be targeted for degradation by chaperone complexes that interact either directly or indirectly (via adaptor proteins) with the ubiquitination machinery. Similar coupling of chaperones to proteases is likely to be involved in the QC of other organelles (e.g. mitochondria) where proteins undergo maturation. Thus, there is an intimate and reciprocal relationship between protein maturation and QC, with chaperones playing key roles in both.

In addition to the biosynthesis steps, other layers of QC are also employed by the cell. Intracellular trafficking by sorting and transport factors often require that proteins be folded into their native configuration to allow their recognition as suitable cargo. Furthermore, additional factors in the Golgi are also thought to be competent in the exclusion of damaged or misfolded proteins from further trafficking along the secretory pathway and instead reroute them for degradation in lysosomes. Similar organelle-specific QC components have been described in other cellular compartments such as the nucleus and mitochondria, which target the non-native proteins for degradation in the cytosol and elsewhere (e.g. in the mitochondrial matrix).

The amount of substrate flux through the various QC pathways is also monitored by the cell. If QC capacity is approached or exceeded, the cell has *stress response* mechanisms that signal the need for more biosynthetic, maturation, QC and degradation components. Stress response pathways typically operate in modular (albeit somewhat overlapping) fashion such that need in one compartment (e.g. the ER) is accompanied by a predominantly ER-selective response that upregulates only those components needed for ER function and QC. Two of the most well-studied responses are the unfolded protein response (of UPR) initiated from the ER, and the heat shock response typically operating from the cytosol (Morimoto, 1998; Ron and Walter, 2007). In both cases, the sensing mechanism is thought to involve the titration of key chaperone(s) by excess substrates. Thus, changes in the demand for maturation and QC machinery in any cellular compartment can be quickly met by an appropriate response.

Part II: Consequences of Aberrant Proteins

The cell clearly has many mechanisms of biosynthesis, trafficking and QC to both minimize and deal with aberrant proteins. Furthermore, physiologic stress response pathways can alleviate and compensate for changes in substrate load on these pathways. Thus, the pathways to maintain protein homeostasis in the cell under various conditions are both robust and redundant. What are the limits of these pathways, and under what circumstances are these limits exceeded? And what are the consequences of excessive aberrant proteins for cell physiology? To address these questions, it is instructive to look at examples where aberrant proteins are generated in a defined, well-established manner: inherited mutations in a specific gene. The diseases caused by such mutations are often rare, but they provide the opportunity to investigate the consequences of aberrant proteins (i.e. the development of a specific disease state) under circumstances where the root cause is well established. Only with a clear understanding of potential mechanisms can one apply the principles to what various multifactorial processes such as aging, in which aberrant protein metabolism is often implicated but whose causes and consequences remain mysterious.

Genetic mutations that give rise to aberrant proteins could impact any of the steps of synthesis, maturation, trafficking and degradation (**Figure 2**). In general terms, any such deviations from the norm would lead to at least some loss of the protein to degradative pathways and hence a loss of its normal cellular function (**Figure 2b**). In addition, the fact that the mutant protein necessarily has a different (even if only slightly in some cases) trafficking or metabolic pathway than normal means that it has the *potential* to interact with molecules ordinarily not seen by the wild-type protein. Thus, mutant proteins can sometimes acquire properties or functions in the cell that are distinct from the normal one (**Figure 2c**). Either of these scenarios could contribute to the downstream injurious effects on the health of the cell and progression to disease. Importantly, the two routes of adverse consequences are not mutually exclusive, and it is likely that the phenotype of many diseases are a complex combination of both a loss of normal function and gain of additional properties by the aberrant protein. For simplicity, however, the discussion below will treat these mechanisms of disease separately.

Loss of function inherited disorders

If an inherited mutation results in an aberrant protein that is recognized and targeted for degradation by QC pathways, it then leads to a loss of that protein's cellular function. Cystic fibrosis (CF) is considered as a classic loss of function disorder. This disease is a result of inherited mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR), a plasma membrane chloride ion channel (Gadsby *et al*., 2006). A single amino acid deletion at position 508 of this protein (ΔF508) is the most common (and most extensively studied) mutation associated with disease and results in misfolding and rapid proteasomal degradation of CFTR (Turnbull *et al*., 2007). The deficiency of CFTR and therefore loss of its chloride channel function leads to inappropriate hydration of the secretions of cells that line organs and results in the obstruction of airways and glands by

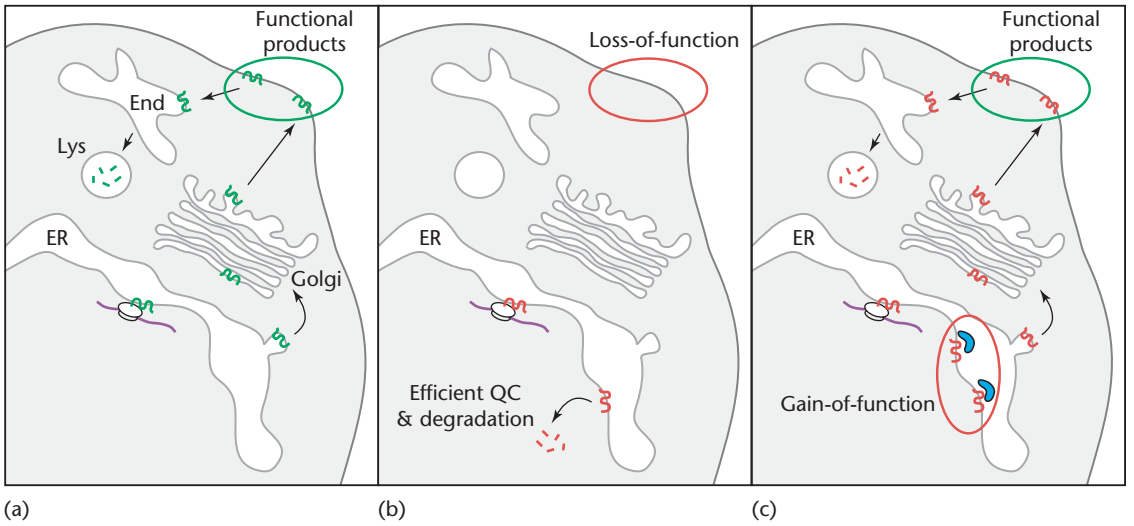

Figure 2 Loss versus gain of function mechanisms. (a) Schematic depiction of the biosynthetic and degradation pathways of a cell surface membrane protein (green) that is initially made at the ER, trafficked through the Golgi to the cell surface, and eventually degraded in the lysosome. (b) In a purely loss of function mechanism, an aberrant version of this same protein (red) would be efficiently recognized and degraded by the cell. Cellular dysfunction is due solely to the absence of the protein. (c) In a gain of function mechanism, the aberrant protein would interact inappropriately with and influence the function of cellular factors not typically encountered by the normal version. In this example, retention of the aberrant protein in the ER allows it to interact with a resident protein (blue) whose altered function is the cause of cellular dysfunction.

mucus. Retention of pathogens (especially bacteria) in the thick mucus leads to chronic infections and lung disease that is often the primary cause of mortality in CF patients. **See also**: Cystic Fibrosis

Remarkably, ΔF508 CFTR is misfolded in a very subtle manner in only one region on the cytosolic face of this protein. Whereas this is sufficient for its recognition by QC and/or prevention of its normal trafficking, it does not disrupt function completely. Indeed, escape of this protein from QC can actually lead to functional rescue in cultured cells. However, unless highly specific to CFTR, suppressing QC is unlikely to represent a reasonable therapeutic strategy since the consequent escape of other aberrant proteins is almost certain to be highly detrimental over time. Nonetheless, CFTR does illustrate that loss of function can occur in any of a number of ways that do not necessarily lead to a nonfunctional protein per se. Numerous genetic diseases appear to be caused primarily, if not exclusively, by a mutation that affects the respective protein's maturation or trafficking such that it is effectively nonfunctional due to its efficient routing for degradation.

Diseases that act primarily by a loss-of-function mechanism are almost always recessive, as mutation of both alleles is necessary to lead to a complete loss of function. These disorders therefore mimic a null phenotype for that gene, as the aberrant proteins are dealt with highly effectively by QC pathways. The mutant protein in these diseases does not make inappropriate interactions with other cellular components that could lead to detrimental effects for the cell. For instance, while ΔF508-CFTR is recognized as aberrant by the QC machinery and retained within the ER, there is no evidence that the mutant protein causes any additional problems for the cell before its rapid degradation. Indeed, studies in mice have illustrated that a clean knockout of CFTR has essentially the identical phenotype as mice carrying a homozygous ΔF508 mutation.

Gain of function inherited disorders

Mutant proteins are not always recognized and degraded efficiently by QC pathways. This failure of QC can have several consequences for the cell that in some cases are far worse than if the protein were entirely absent. This is because the mutant protein, or a metabolic product generated from it (e.g. a peptide fragment), has a novel function or activity (often totally unrelated to its normal function) that is detrimental to the cell. Therefore, genetic mutations that impart a new or aberrant property on the protein to cause cellular dysfunction are termed *gain-of-function* (GOF) diseases. GOF diseases typically show dominant inheritance because the mutant protein from one allele is often sufficient to impact cellular function. As the pathways of QC and degradation are complex and occur in multiple cellular compartments, there are numerous ways in which GOF could manifest (**Figure 3**). In general, the mutant protein interacts inappropriately with cellular factors that the normal protein either does not interact with at all, or in some cases, in a different way than the normal protein.

Several well-studied examples of dominant GOF diseases show the accumulation of aggregated mutant protein either intra- or extracellularly. This is the case in a set of disorders termed polyglutamine expansion diseases (Ross, 2002). In these diseases, a gene that normally has a stretch of many glutamine codons is mutated during its replication to encode substantially more glutamines. This expanded polyglutamine repeat (PolyQ), if greater than

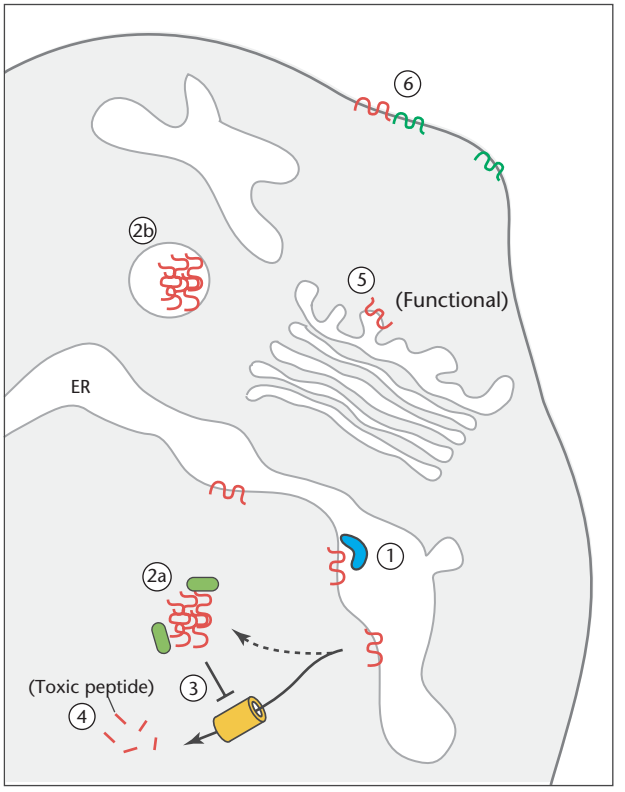

Figure 3 Multiplicity of toxic mechanisms by an aberrant protein. Dominant gain-of-function interactions by an aberrant protein (red) can occur in many ways with different partners to cause cellular dysfunction. Most diseases are likely to involve multiple interactions and multiple mechanisms, perhaps explaining their complexity. Some examples are: inappropriate interactions due to altered conformation or residence in an incorrect location (1); failure to be degraded efficiently, generating aggregates that sequester factors (2a) or inhibit organelle function (2b); interaction with and inhibition of QC or degradation machinery (3); generation of metabolites that are toxic (4); performing its function at an incorrect location (5) and interaction with and inhibition of the normal version of the same protein (green) (6).

approximately 40 residues, can lead to disease in a length-dependent manner. Huntington disease (HD) is a very well-studied member of this subset of diseases and is characterized by the synthesis of the protein Huntingtin (Htt) containing an expanded PolyQ region. Htt containing an expanded polyQ, unlike normal Htt, seems to be degraded less efficiently by the proteasome. Instead, mutant Htt has a higher propensity to misfold and aggregate in the nucleus (preferentially) and cytosol. Thus, polyQ-expanded Htt can differ in several ways from its normal counterpart: it persists in the cell for a longer time, can form self-associated products that range from small oligomers to large aggregates and can interact with various components of the biosynthetic, QC and degradation machinery normally charged with its metabolism. At least some of these new properties are thought to cause disease since lacking the mutant allele altogether (and just containing one normal allele) is well tolerated in mice. **See also**: Huntington Disease; Protein Aggregation and Human Disorders; Trinucleotide Repeat Expansions: Disorders

Precisely, *how* mutant Htt (or any aggregate-forming protein) leads to cellular dysfunction remains a matter of intense study and considerable controversy. One mechanism might involve the considerable burden placed on the QC and degradation pathways by disassembling and/or preventing protein aggregates. In addition, mutant Htt and aggregates generated from it have been shown to interact with and possibly sequester various cellular proteins including nucleoporins, transcription factors, cell cycle components, chaperones and the ubiquitin-proteasome machinery. By preventing small proportions of these other cellular proteins from performing their normal function, Htt is posited to cause the downstream neuronal death and cognitive changes seen in disease. Thus, an aberrant protein (Htt in the above example) undergoes a series of interactions with the QC machinery and other pathways during the course of its metabolism. Such interactions are necessarily different from the normal version, either qualitatively (e.g. entirely novel interactions) or quantitatively (e.g. an abnormally strong or lengthy interaction with a normal partner). This can have different consequences at each of the steps that can potentially contribute to the overall phenotype. The same basic principle applies to many other aberrant proteins, although the specific QC and metabolism machinery vary. The important point, however, is that the altered pathway taken by the aberrant protein can lead to inappropriate interactions, with implications for various cellular processes.

In most of these diseases, how and why a proportion of these mutant proteins bypass the QC machinery is incompletely understood; however, the cell-type-specific cytotoxicity often seen in these diseases argues that QC components of different cell types may be differentially permissive of different types of aberrant proteins. Perhaps their respective capacities or substrate ranges, determined by the ensemble and levels of chaperones, are different. Alternatively, the relative importance of the intersecting pathways affected by the aberrant protein may vary in a cell-type selective manner. For instance, perhaps sequestration of a specific protein is more damaging to certain cell types due to the absence of homologous proteins with redundant functions. It is worth noting that the phenotype of these GOF diseases does not necessarily provide insight into the normal function of the mutated gene. In fact, numerous GOF diseases are associated with proteins whose normal function remains largely obscure. In HD, although knockout of the HD gene in mice is embryonic lethal, the function of the protein is not yet precisely understood. Htt has been implicated in a variety of functions including vesicular trafficking, calcium signalling and transcriptional de-repression, but whether any of these functions are related to the phenotype is debatable.

In some GOF diseases, the aberrant protein may act in a dominant-negative fashion by interacting with and inhibiting the function of its normal counterpart encoded by the wild-type allele. One example of this can be seen in osteogenesis imperfecta, a dominantly inherited brittle bone disease caused by mutated type I collagen, an extracellular

matrix protein. Collagen is a triple helical fibril composed of two α1 chains and one α2 chain. A genetic mutation in one copy of either of these 2 genes leads to the formation of an aberrant collagen fibril with altered matrix-to-cell signalling and compromised structural integrity of the extracellular matrix. In this way, the aberrant chains can alter the functional properties of the normal protein, leading to a dominantly inherited GOF disorder. Clearly, these effects require that the mutated collagen chain at least partially escape QC pathways in the ER to reach the extracellular environment. How or why this occurs remains unknown, highlighting again the importance of understanding the basic mechanisms of cellular QC pathways.

Nongenetic sources of aberrant proteins

The examples cited earlier illustrate the general mechanisms that are in play in inherited disorders of aberrant proteins. Although genetic mutations provide a uniquely convenient paradigm for aberrant proteins, other causes such as environmental toxins, nutrient deficiencies or excess, oxidative stress, heat or even intrinsic inefficiencies are a far more common source. The effects of these nongenetic factors tend to be very widespread as they simultaneously affect a variety of different proteins and pathways in the cell, thereby leading to pleiotropic effects. Even in well-defined experimental systems such as heat stress of cultured cells, the effects are numerous: increased protein misfolding, altered efficiencies of various cellular reactions, titration of chaperones, protein aggregation, oxidative stress and so on. Hence, the exact reasons why some cells succumb to its consequences versus adapt successfully are difficult to identify. Additionally, different cell types are differentially sensitive to the same stressors, emphasizing that their relative capacities to deal with aberrant proteins or the downstream consequences of their accumulation vary considerably. This is likely one reason why nongenetic disorders associated with aberrant protein production or metabolism are exceedingly difficult to understand at a mechanistic level, and why so many different aetiologies have been reported for any one disease. For example, elevated levels of an essential nutrient such as iron can lead to oxidative stress, protein damage and many physiologic consequences in various tissues. Indeed, iron overload has been implicated in many diseases including neurodegeneration, but the exact mechanisms remain unknown. Nonetheless, the root causes of many such disorders may well lie in the *cumulative* effects of numerous subtle inappropriate interactions that arise as a consequence of altered maturation, QC or trafficking of multiple individual proteins. With the exception of highly selective toxins, it is presumably rare for a nongenetic aetiology of aberrant proteins to initially affect only one (or a small number) of proteins.

One particularly dramatic example of an acquired disorder that does impact a single gene product is the transmissible prion diseases. These fatal neurodegenerative diseases are a result of aberrant metabolism of the prion protein (PrP): a ubiquitously expressed cell surface protein of unknown function (Prusiner, 1998). In rare circumstances, PrP can acquire an aberrant conformation, termed PrP^{Sc}, that has a unique property: it can interact with and cause the refolding of normal PrP into another copy of PrP^{Sc}. Over time, this leads to the amplification of PrP^{Sc}, whose relatively slow turnover leads to its accumulation. Accumulated PrP^{Sc}, via pathways that remain unknown, leads to neurodegeneration and death. PrP^{Sc} from one individual can then be acquired by another (via ingestion, contamination of transplanted tissue or other means), where it can initiate the same events culminating in fatal neurodegeneration. Thus, the transmissible prion diseases represent a novel category of aberrant protein disorders that are acquired (and transmitted) via a misfolded protein that alters the metabolism and trafficking of its normal cellular counterpart. **See also**: Prion Disorders

Multiple routes to cytotoxicity and the importance of context

As discussed at the outset, a rigid dichotomy of loss- versus gain-of-function mechanism is overly simplistic for most diseases caused by aberrant proteins. The loss of a protein from a complex organism does not have consequences for every cell that ordinarily expresses that protein; in addition, expression of an aberrant protein with potential gain-of-function activities will have different effects on different cell types. In other words, context matters when considering the effects of aberrant protein expression and nearly all diseases are likely to involve multiple mechanisms of cellular toxicity. There are several reasons for this, some of which have already been alluded to earlier.

Many proteins are part of larger families of homologous or orthologous proteins that have similar functions. Hence, the loss of function of any given protein can potentially be compensated by another member of the same family, thereby alleviating the phenotype. Since such functional redundancy may not occur in all cell types at all times (e.g. if the homologous protein has a different expression pattern), the consequence of an aberrant protein that typically acts by a loss of function can vary. In addition, an aberrant protein which is harmlessly disposed off in one cell type might pose special problems for the QC machinery in another, leading to *selective* gain of function effects.

This is because the maturation, QC and degradation machineries vary substantially from one cell type to another. This is seen in many ways. For example, highly secretory tissues have ER containing extremely high levels of chaperones. Other cells may contain certain unique chaperones or maturation factors needed for specialized purposes or for cell-type selective substrates. Thus, different compartments of different cell types are unique in not only their physicochemical environment (redox state, pH, macromolecular composition), but are optimized in different ways for the proteins that reside, mature or transit through them. This variable composition means that QC pathways are likely to differ both in the mechanisms and efficiencies by which they recognize any given proteins as non-native,

and how rapidly it is trafficked or eliminated from the cell. These differences are likely to contribute to the cell selective phenotypes often seen in protein misfolding diseases. In these and related ways, the net effect in a complex multicellular organism may be highly nuanced and multifactorial, with some cell types displaying loss of function effects, others displaying gain-of-function effects, and yet others displaying both.

This concept is perhaps best illustrated by genetic disease caused by mutant of α1-antitrypsin (α1AT), a serum serine protease inhibitor that is secreted by hepatocytes to counteract the effects of damaging neutrophil proteases such as elastase in the lungs (Perlmutter, 2006). The most common cause of α1AT deficiency is a homozygous missense mutation (E342K) that leads to protein misfolding, retention in the ER and subsequent degradation by the proteasome. This classical loss of function disorder is recessively inherited and leads to emphysema in affected individuals due to unrestrained digestion of pulmonary connective tissue by elastase. Interestingly, in a subset of patients with this mutation, the cause of mortality is liver disease rather than emphysema and is due to the selective accumulation of polymeric misfolded α1AT in hepatocytes. This accumulation leads to cytotoxicity by triggering apoptotic and authophagic pathways. Hence, in this disease the same mutation can progress by either loss or gain of function mechanism, depending on the tissue type in question (lung versus liver, respectively), suggesting that tissue-specific factors often contribute to the progression of all misfolding diseases. Therefore, our understanding of the mechanisms of pathogenesis in several diseases continues to evolve, and multiple routes to cytotoxicity caused by aberrant proteins are likely to emerge as a rule, rather than the exception.

See also: Antitrypsin (AAT) Deficiency-α_1

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