REVIEW ARTICLE

Why is ALS so Difficult to Treat?

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ABSTRACT: Amyotrophic lateral sclerosis (ALS) is proving intractable. Difficulties in pre-clinical studies contribute in small measure to this futility, but the chief reason for failure is an inadequate understanding of disease pathogenesis. Many acquired and inherited processes have been advanced as potential causes of ALS but, while they may predispose to disease, it seems increasingly likely that none leads directly to ALS. Rather, two recent overlapping considerations, both involving aberrant protein homeostasis, may provide a better explanation for a common disease phenotype and a common terminal pathogenesis. If so, therapeutic approaches will need to be altered and carefully nuanced, since protein homeostasis is essential and highly conserved. Nonetheless, these considerations provide new optimism in a difficult disease which has hitherto defied treatment.

RÉSUMÉ: Pourquoi la SLA est-elle si difficile à traiter? : La sclérose latérale amyotrophique (SLA) s'avère impossible à traiter. Les difficultés rencontrées dans les études précliniques contribuent en partie à cet insuccès. Mais la principale raison d'échec est liée au fait que la pathogenèse de la maladie demeure mal comprise. Plusieurs mécanismes acquis ou héréditaires ont été proposés comme causes potentielles de la SLA, mais bien qu'ils puissent conférer une prédisposition à la maladie, il semble de plus en plus plausible qu'aucun ne mène directement à la SLA. Il semble plutôt que deux théories qui se chevauchent, qui toutes deux impliquent une homéostasie protéique aberrante, pourraient mieux expliquer l'observation d'un phénotype commun associé à une pathogenèse de base qui serait commune. Si c'est le cas, les approches thérapeutiques devront être modifiées et nuancées avec précaution parce que l'homéostasie protéique est essentielle et hautement conservée. Néanmoins, ces considérations fournissent un nouvel optimisme face à une maladie pénible qui est demeurée intraitable jusqu'ici.

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No neurodegenerative disease is well treated, but Amyotrophic lateral sclerosis (ALS) is proving particularly intractable. To date, 53 of 54 potential treatments tested in human trials (Table 1)[1-57] have failed and the sole successful agent (riluzole) is supported by evidence so muted that false positive results due to chance cannot be completely excluded. Indeed it is likely that additional failed trials have gone unreported. The following discussion, hypothetical by nature, sets out possible reasons why therapeutic success has been elusive to date, outlines two recent directions that may be important in our understanding of ALS, and suggests reasons why future treatment may continue to be challenging.

A. Why has therapeutic success been so difficult to date?

ALS is a complex disease with genetic and environmental components

First, ALS has been difficult to conceptualize and treat because important aspects are incompletely understood. Sporadic and multiple familial forms are recognized that are clinically indistinguishable[58], but we do not understand the explanation for the common disease phenotype. The only predisposing factor of major significance is age, for reasons that also remain unclear.

Multiple cell types are involved, with genetic, environmental, and possibly stochastic influences at play. The chief features of ALS relate to motor neuron involvement with variable frontotemporal dementia, but multiple cell types modify disease expression. In transgenic mice over-expressing a human mutant SOD1 (mSOD), the first-described cause of familial ALS[59] (FALS), restricting expression of mSOD to motor neurons alone may or may not result in any clinical disease[60-62], and the disease is modified by transgene expression in microglia[63], oligodendroglia[64], astroglia[65], Schwann cells[66,67], and skeletal muscle[68]. Even within the same cell type, there is important heterogeneity[69,70].

Twin studies in sporadic ALS have identified the likelihood of genetic and environmental influences, perhaps of about equal magnitude[71]. Environmental influences could include neurotrophic viral infection, neurotoxins, and nervous system trauma,

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Table 1: Agents studied in human ALS trials

Agent	Rationale	Result	Reference
TCH 346	Anti-apoptotic	negative	[1]
Physostigmine	Anti-cholinergic	negative	[2]
Tet hydroaminoacridine	Anti-cholinergic	negative	[3]
Branched chain AAs	Anti-excitotoxic	negative	[4]
Ceftriaxone	Anti-excitotoxic	negative	[5]
Dextromethorphan	Anti-excitotoxic	negative	[6]
Gabapentin	Anti-excitotoxic	negative	[7]
Lamotrogine	Anti-excitotoxic	negative	[8]
L Threonine	Anti-excitotoxic	negative	[9]
Memantine	Anti-excitotoxic	negative	[10]
Nimodipine	Anti-excitotoxic	negative	[11]
Talampanel	Anti-excitotoxic	negative	[12]
Topiramate	Anti-excitotoxic	negative	[13]
Transcranial stim.	Anti-excitotoxic	negative	[14]
Verapamil	Anti-excitotoxic	negative	[15]
Riluzole	Anti-excitotoxic ?	positive	[16]
Valproic Acid	Anti-excitotoxic....	negative	[17]
Azothiaprine	Anti-inflammatory	negative	[18]
Celecoxib	Anti-inflammatory	negative	[19]
Cyclophosphamide	Anti-inflammatory	negative	[20]
Cyclosporine	Anti-inflammatory	negative	[21]
Glatiramer Acetate	Anti-inflammatory	negative	[22]
Interferon alpha	Anti-inflammatory	negative	[23]
Interferon beta	Anti-inflammatory	negative	[24]
IvIg	Anti-inflammatory	negative	[25]
Minocycline	Anti-inflammatory	negative	[26]
Pentoxyfilline	Anti-inflammatory	negative	[27]
Snake Venom	Anti-inflammatory	negative	[28]
Total lymph radiation	Anti-inflammatory	negative	[29]
Edaravone	Anti-oxidant	negative	[30]
Glutathione	Anti-oxidant	negative	[31]
n-Acetyl Cysteine	Anti-oxidant	negative	[32]
Selegiline	Anti-oxidant	negative	[33]
Vitamin E	Anti-oxidant	negative	[34]
Selegeline + Vitamin E	Anti-oxidant	negative	[35]
Amantadine / guanidine	Anti-viral	negative	[36]
Guanidine	Anti-viral	negative	[37]
Indivavir	Anti-viral	negative	[38]
Isoprinosine	Anti-viral	negative	[39]
Tilorone	Anti-viral	negative	[40]
Lithium	Autophagy / mitochondrial	negative	[41, 42]
Transfer factor	Immunomodulator	negative	[43]
CoEnzyme Q10	Mitochondrial	negative	[44]
Creatine	Mitochondrial	negative	[45]
Dexpramipexole	Mitochondrial	negative	[46]
Olesoxime	Mitochondrial	negative	[47]
BDNF	Neurotrophic	negative	[48]
Ganglioside	Neurotrophic	negative	[49,50]
Growth Hormone	Neurotrophic	negative	[51,52]
Insulin-like GF (IGF)	Neurotrophic	negative	[53]
Xaliproden	Neurotrophic	negative	[54]
CNTF	Neurotrophic	negative	[55]
TRH	Neurotrophic ?	negative	[56]
3,4 Diaminopyridine	transmitter release	negative	[57]

as set out later. There may be a genetic/environmental interaction in ALS that obscures epidemiological attempts to identify these components separately, and further complicates an already difficult disease.

In highly inbred transgenic mSOD mice, there is still variability in disease onset that might reflect differences in unrecognized genetic modifiers or, perhaps just as likely, reflect the play of chance.

Preclinical studies are problematic

Nearly all agents that have failed in recent human trials have been supported by positive preclinical studies in transgenic mice over-expressing a human mutant SOD1. It is possible that mSOD mice imperfectly model sporadic and most familial ALS except mSOD FALS, and even here, animal models may imperfectly mirror neurodegenerative disease in humans where the regenerative capacity of the central nervous system (CNS) is so limited.

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In mouse studies the experimental agent can be (and usually has been) administered before disease onset which may be more applicable to disease prevention rather than treatment, as discussed below. Also, transgenic ALS mice are more homogeneous than humans, in whom the cause of disease is mostly unknown and the onset and progression highly variable. It can be difficult to extrapolate an optimized dose from mouse to man, and in animals it is easier to establish effective tissue delivery and biological effect at a cellular level (of relevance to failed agents with a tight therapeutic window such as lithium, and growth factors).

However, in spite of all these caveats the positive preclinical results seen in ALS mice have generally been modest for all agents studied, and indeed, usually negative when repeated using more stringent experimental conditions[72].

Thus, it is possible that larger human trials in more selected patients might have shown some benefit, and it is possible that undue reliance on preclinical models may have led to the unwarranted testing of some agents in humans. However, the reasons for failure of ALS therapy in humans run far deeper than either of these possibilities.

ALS has a focal onset and spreads within the neuraxis

Amyotrophic lateral sclerosis is usually characterized by a distinct topographical spread within the nervous system[73], with some areas of the neuraxis uninvolved when other areas are diseased. Perturbation of those diverse balances- excitatory and inhibitory, pro- and anti-inflammatory, pro- and anti-apoptotic, etc, within the normal nervous system may be harmful, and treatments restoring a more beneficial balance in diseased areas risk distorting the balance in uninvolved areas.

A case in point is anti-inflammatory treatment. The symptomatic phases of ALS are accompanied by a deleterious microglial inflammatory response. However, microglia early in the ALS process in transgenic mice are neuroprotective, and only in the later stages do they accelerate neuronal death[74]. Anti-inflammatory treatment could be beneficial in some areas of the neuraxis, neutral in some areas, and harmful in others, with an uncertain effect on the organism as a whole. This may explain, at least in part, why attempts at reducing inflammation have been unhelpful or even harmful (minocycline) in human studies.

A similar neuroprotective/neurotoxic dichotomy exists for astroglia at different stages in the illness[75], with the similar conceptual concerns.

Past and present approaches may be more applicable to prevention than treatment

Multiple metabolic and genetic abnormalities potentially toxic to motor neurons have been discovered in sporadic and familial FALS, and multiple causes of disease thereby suggested. ALS is usually conceptualized as a long-standing disease resulting from progressive cellular abnormalities such as these, exacerbated in the later stages by secondary pathology such as inflammation, eventually leading to the progressive death of motor neurons. However, as set out below, neither the metabolic abnormalities nor the presently known FALS mutations may be the proximal cause of the progressive paralysis of voluntary muscle that is the hallmark of clinical disease, and treatment targeting these processes may be more suited to the prevention of ALS than its treatment.

Metabolic abnormalities associated with ALS do not directly cause ALS

Metabolic abnormalities identified in sporadic ALS include mitochondrial dysfunction, oxidative stress, inflammation, excitotoxicity, axonal impairment and trophic factor deficiency[76]. The treatments outlined in Table 1 are grouped by the putative disease etiology targeted, as above and, for nearly every rationale, multiple agents have been tried in well conducted human trials, some several times each, and all have failed. One can only assume that our presumptions of causality have been overly simplistic and either these abnormalities are not amenable to standard treatment or they are not essential to the actual ALS disease process.

It might be worth pointing out that no one has reported ALS induced in normal mice by treatments disrupting any of these processes. It remains possible that a multifaceted approach (targeting several or all of these) is needed but this lacks strong animal support and has failed when tried in humans[77]. Many processes are under homeostatic control and, if an observed abnormality arises secondary to a more relevant abnormality elsewhere in a feedback loop, correcting the observed abnormality could be counter-productive. Attempts to control transmitter levels may be particularly subject to these effects.

FALS mutations predispose to ALS but do not directly cause ALS

We now recognize more than a dozen diverse mutations that all cause Mendelian FALS, the most relevant outlined in Table 2. However, it is clear that none of these genetic 'causes' directly causes ALS in the usual sense and, specifically, ALS does not arise as an inevitable consequence of perturbations brought about by FALS mutations. Rather, they predispose to ALS and the ALS disease process itself- the rapidly progressive weakness of voluntary muscle- is separate.

All FALS mutations produce a phenotypically similar disease, after a similar asymptomatic period averaging 40-60 years (Table 2) and, once installed, the disease caused by all mutations generally has a progression independent of the trigger[78]. The gene for which we have the greatest information is mutated SOD1, and here different mutations have very different rates of progression. If mSOD directly led to ALS, the end result of a monotonic disease process, those particular mutations of early onset should have the most rapid progression. Figure 1 is derived using data from Cudkowicz *et al*[79], to demonstrate that if anything, the opposite occurs. Much more often than chance alone would allow, some FALS patients harbour two different causative mutations, yet do not differ in disease progression from patients with a single mutation[80].

Transgenic mice differing in the degree of mSOD over-expression show different ages of disease onset depending on the transgene copy number, with earlier onset seen in animals with the higher copy number[81]. However, the degree of over-expression plays no or little role in the progression of clinical disease, which remains relatively constant. In one study high copy SOD1 mice had clinical disease onset at 109 days and

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Table 2: Onset and onset range of most common known ALS mutations (ALS On-line genetics database, available from www.alsod.iop.kcl.ac.uk/)

Name	Mutation	Onset (yr)	Range
ALS 1	Super Oxide dismutase (SOD1)	47	14 - 79
ALS 4	Senetaxin	18	1-73
ALS 6	Fused in Sarcoma (FUS / TLS)	44	11 - 80
ALS 8	VAPB	44	18 - 73
ALS 9	Angiogenin (ANG)	55	21 - 83
ALS 10	Tar Binding protein 43 (TDP 43)	54	30 - 77
ALS 11	FIG4	55	29 - 77
ALS 12	Optineurin (OPTN)	51	24 - 83
ALS 14	Valsolin (VCP)	49	37 - 53
ALS 15	Ubiquilin 2	41	16 - 71
ALS	Dynactin	55	48 - 64
ALS	Neurofilament HC (NFH)	60	46 - 73
ALS	TAF15	50	45-55
ALSFTD 2	C09ORF72	57	?

survived 42 days from that point, while low copy number mice had clinical onset at 380 days, and survived on average 41 days[82].

In asymptomatic FALS carriers destined to develop FALS, evidence of presymptomatic disease is very difficult to demonstrate, at least by sequential motor unit counting and electromyography[83,84]. Generally, symptoms and signs onset roughly co-incident with the electrophysiological abnormalities, with only a slight delay due to collateral sprouting. Furthermore, most recognized FALS mutations are autosomal dominant with incomplete penetrance and, typically, 10-20% of obligate carriers remain disease-free over a normal human lifespan[85,86], although the true percentage may be higher[87].

Finally, in transgenic mice and rats over-expressing mSOD, it is possible to target the mutant protein for destruction, or stop expression of the mutant protein at any given age. Infusion into the cerebrospinal fluid (CSF) of anti-sense oligonucleotides targeting G93A SOD1 mRNA did not arrest disease in mSOD mice, and prolonged survival by about 8%[88]. Chemically stabilized siRNA targeting mSOD1 mRNA infused into the CSF shifted the survival curve in mSOD mice, but only modestly[89]. Infusion of antibodies targeting the G93A SOD1 protein prolonged survival by about 4%[90]. When expression of mSOD was reversed in conditional expression models, disease progression was slowed, but all animals progressed and died nonetheless[91]. These results suggest that reducing or eliminating exposure to mutant mSOD after disease onset might slow disease progression, but either the experimental interventions were unsatisfactory or a separate and at least partially independent process had been triggered.

B. ALS may be a separate process of disrupted proteostasis.

In summary, neither neurotoxic abnormalities associated with ALS, nor presently known FALS mutations, directly cause ALS, and the major reason ALS is untreatable is that key aspects of the disease remain misunderstood. Clearly the ALS phenotype has additional and unique pathophysiologic mechanisms. Understanding these mechanisms is no guarantee of successful treatment but, in the absence, lack of success is not surprising.

Amyotrophic lateral sclerosis may be a distinct and predominantly neuromuscular process that arises with greater probability in the presence of predisposing cellular disease (metabolic perturbation, FALS mutation, neurotropic virus, etc), and, once triggered, might be self-perpetrating and capable of spread, rapidly progressive and less dependent on the initial trigger. We need then to understand how a rapidly progressive process could arise suddenly on a background of presumed good health (in sporadic disease) or, in FALS, on the background of a genetic mutation with metabolic perturbation present since conception. There are several ways this concerning scenario might arise, and two inter-related processes seem most likely to be central. Unfortunately, both involve perturbations to the

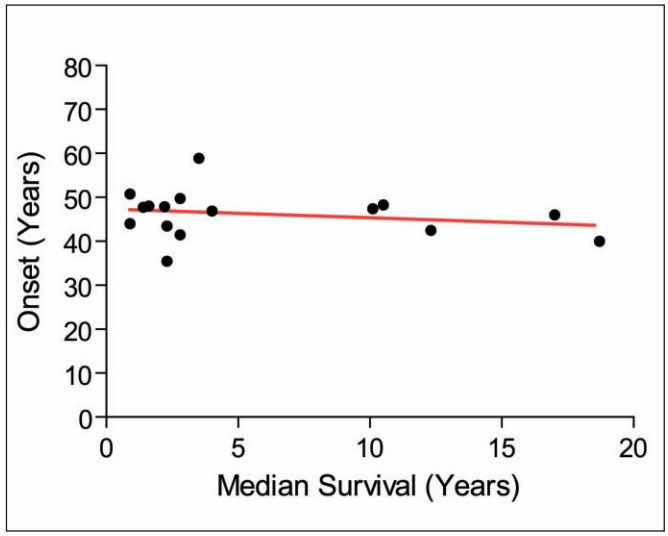

Figure 1: *Onset and survival of 16 different human SOD1 mutations (after Cudkowicz[79]).*

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highly conserved process of protein homeostasis ('proteostasis'), and treatment considerations may need to be considerably nuanced.

i. Prion-like proteins contribute to ALS, but are linked to essential cell function

Amyotrophic lateral sclerosis shares many features with classical prion disease and, although there is no evidence that ALS is transmissible in the usual sense[92,93], abnormal proteins with prion-like qualities accumulate in ALS and *in vitro* can spread from cell to cell[94,95]. This cell-to-cell transmission is conceptually attractive as it could underlie the topographic spread of disease characteristic of many patients.

Misfolded SOD1 may behave as a self-templating protein in ALS[94,95]. As would be expected, over-expression of wildtype (WT) SOD1 accelerates disease in mSOD transgenic mice[96], and reducing WT SOD1 delays disease onset and slows progression in mSOD mice[97,98]. However, most of the conceptual interest lies in prion-like proteins involved in RNA processing.

Using bio-informatics algorithms, it is possible to predict the propensity of any protein to misfold, and of 21,873 human genes analyzed in this way, 246 were predicted to produce proteins with prion-like domains, and of these, the small group of RNA binding proteins were twelve-fold over-represented[99]. There are now six such proteins linked to ALS (TDP43[100,101], FUS/TLS[102,103], TAF15[104,105], EWSR1[106], hnRNPA2B1[107], hnRNPA1[107]), with undoubtedly more to follow. (hnRNP refers to heterogeneous nuclear ribonucleoprotein, the class name for all these RNA binding proteins). The predicted prion-like regions of these proteins are distinct from the RNA Recognition Motif (RRM) regions, yet toxicity requires both[108,109]. Thus, it is unlikely that toxicity flows from the simple accumulation of poorly-digestible prion-like protein fragments in the cell.

Indeed, it is unlikely that the propensity of RNA binding proteins to 'misfold' has evolved by happenstance, and it has been suggested that RNA binding proteins co-aggregate physiologically in cytosolic mRNA processing bodies (P bodies) and stress granules through their alternative conformational region[110]. Thus, the protein misfolding may not be an unfortunate evolutionary accident but rather a conserved function essential to normal protein homeostasis.

Familial ALS mutations occurring in the prion-like regions of RNA binding proteins serve to increase the propensity of these proteins to aggregate through their prion-like domains[107,111]. This may unduly stabilize stress granules and P bodies so that they no longer disaggregate when appropriate, progressively sequestering mRNAs and miRNAs and other RNA binding proteins in the cytosol to the detriment of normal mRNA processing. Conceptually, the presence of more than one RRM on the same RNA-binding protein (e.g. TDP43 has two[99]) might allow more complex aggregates to occur.

TDP43 and FUS/TLS at least are also mRNA transport proteins[112], found in dendrites and axons[113,114], and local protein synthesis could be impaired if these transport proteins were inappropriately retained in cytosolic aggregates. Interference with distal axonal protein production would be of particular interest in ALS given the susceptibility introduced by the length and activity of motor axons. Last, RNA binding proteins play essential roles in the nucleus (facilitating, *inter alia*, the splicing of pre mRNA) and toxicity could also flow from disrupted nuclear processing of mRNA[115].

As one would expect, there are physiological controls on RNA binding proteins that serve to prevent pathological aggregation. In yeast, the parent mRNA of these proteins is present in low quantities, the translation efficiency is low, and the proteins themselves have short half-lives[116]. TDP43 at least controls its own expression level (in part by binding to and de-stabilizing its mRNA transcript[117]), and sequestration in disease states could increase translation and fuel cytosolic aggregation. In keeping with this, over-expressing human wildtype protein results in a dose-dependent reduction in endogenous mouse TDP43 mRNA[118]. Beyond a certain threshold the tendency for peripheral accumulation of aggregates in a cell might increase, with aggregation-prone mutations reducing this threshold.

Exchange of cytosolic proteins from an involved neuron to a neighbour could spread disease aggregates in a prion-like fashion. (It has recently been reported that microparticles containing mRNA and associated proteins are present in human CSF, differ between brain-injured patients and controls, and differentially perturb RNA processing in stem cells *in vitro*[119]). This might explain the topographic spread of disease. Cytosolic mislocalization of wtTDP43 is seen in most sporadic ALS patients[100], not just mTDP43 FALS, suggesting that disruption of RNA processing may be widely important.

ii. The unfolded protein response and cellular stress

Intercellular passage of prion-like proteins can occur *in vitro*, but it is unknown whether this process is important *in vivo*, and it is unclear that it would explain the temporal profile of ALS without invoking additional processes. Also, it would seem unlikely that cytosolic sequestration of a single or a few mRNA species could be solely responsible for disease expression. TDP43 binds to over 6000 mRNAs[115], and FUS a smaller number of seemingly different mRNAs[120], yet mutations in either cause the same phenotype. A more general disruption of protein homeostasis seems likely, although the possibility of additional protein-specific effects remains. A wide-spread dysfunction of proteostasis related to a FALS mutation as above, or other processes as below, could trigger cellular stress responses, which are known to occur in ALS.

By way of background, damaged proteins and organelles are targeted for breakdown and recycling by the ubiquitin-proteasome system (smaller and shorter-lived substrates) or autophagy (larger aggregates or organelles). However, protein homeostasis also requires general control of protein production that can respond to environmental factors such as the availability of nutrients, cell injury, or cell cycle state, and can direct cell growth, or cell division, or invoke conservative defense responses. These higher order controls are integrated with regulatory controls in the endoplasmic reticulum (ER) protein secretory pathways that are important in limiting protein misfolding (hence 'unfolded protein response' or UPR). However, the reach of the UPR may extend far beyond the ER, to non-secreted protein control, lipid and glucose metabolism, cell division, innate immunity, the induction of autophagy, and cellular de-differentiation and programmed cell death[121]. As expected, proteasomal function, autophagy, and the UPR are linked[122].

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The UPR is often over-simplified as having three arms, each capable of interacting with other pathways[121]. One arm involves the activation of the endonuclease inositol-requiring protein 1 (IRE1) leading to a splice excision in X-Box1 (XBP1) mRNA and expression of XBP1(s) genes, to increase among others chaperone proteins to assist in protein folding; one arm reduces the production of most other proteins presumably to offload the ER; and the third arm activates the (usually) pro-apoptotic translation factor ATF6, presumably reflecting at that point a degree of protein misfolding incompatible with cell survival. The response is contingent on the cell state, the three arms may act independently, and each may be cytoprotective or pro-apoptotic under different circumstances[121].

The second arm of the UPR is particularly interesting. In eukaryotes, mRNA translation requires the initiation factor Eif2α. When the UPR is activated, specific kinases (in particular, 'PERK') phosphorylate Eif2α and, in its phosphorylated state Eif2α-P is no longer able to initiate most protein translation. (Translation of those proteins needed to combat ER stress may proceed without need for Eif2α initiation[123].) The generalized reduction in translation offloads the ER and allows proper protein folding to catch up.

An important study by Saxena[69] in mSOD mice indicates how a UPR might arise on the background of a FALS mutation that has been present since conception, and contribute to disease. In motor neurons known to be affected early, there is an initial ubiquitin response and upregulation of cytosolic proteasomal function, then an abrupt down regulation of these processes and an almost ten-fold increase in the UPR, conjoined with a marked microglial expansion and activation, that occurs over a four week period just prior to clinical disease onset. In resistant motor neuron pools known to be affected later in ALS, a similar increase in the UPR is seen, later, just prior to their known involvement.

The cause of the sudden transition and UPR spike is unclear, as *a priori* one might have expected persisting proteosomal activity and a slow but steady rise in the UPR. Nonetheless, this spike in the UPR has a temporal profile which might be expected in ALS. Elevated levels of Eif2α-P are present in ALS[124] suggesting that the UPR is operative in human disease.

Prion-like aggregates and the UPR could be tightly linked

Cytosolic mRNA aggregates and the UPR have been presented above as two related processes affecting proteostasis. In reality, a clear distinction between the two may not be possible. Sequestration of mRNAs and RNA binding proteins in abnormal aggregates will activate the UPR, and phosphorylated Eif2α and non-translated mRNAs induced by the UPR are linked to the formation of stress granules[125]. Conceptually, pathological aggregates might afford a better explanation for cell-to-cell spread, while the UPR provides a better explanation for the terminal cell death and common disease phenotype seen in ALS.

Disrupted proteostasis accommodates other features of ALS

Disrupted proteostasis could provide an explanation for the intercellular spread of pathology and the common terminal disease phenotype and, in addition, accommodates several other known or presumed features of ALS. In particular, disrupted proteostasis can be potentiated or triggered by environmental influences and aging and could provide an explanation for the terminal inflammation, as follows.

Disrupted proteostasis and environmental influences

In transgenic mouse models of ALS it is necessary to express the human transgene at high copy number to produce disease. In humans with single copy FALS mutation (the usual state), disease is delayed for 40-60 years on average, but the variability in onset is large (20-80 years; Table 2) and the disease penetrance is incomplete over a normal human lifespan, perhaps because most fully penetrant mutations would be incompatible with survival. Environmental factors could provide a needed second hit, and presumably they are of greater importance in sporadic disease. (Even in 'sporadic' disease there may be undiscovered Mendelian mutations of low penetrance, *de novo* mutation or polygenic influences, and the distinction between familial and sporadic disease may not be clear-cut).

It has been known for several years that sciatic nerve injury in mSOD mice accelerates the disease course[126]. In the above-mentioned study by Saxena[69], the UPR is induced precociously by sciatic nerve crush, suggesting one mechanism whereby genetic and environmental influences interact at the level of protein homeostasis. A single sciatic crush leads to a reversible UPR, but repeated crushes are not reversible. Axotomy of retinal ganglion cells induces a UPR and cell death through apoptosis[127]. As an anecdotal aside, perhaps like most ALS physicians, I have seen several patients whose disease followed temporally and spatially regenerative trauma to the same region, and in light of the above, such occurrences may not be coincidental.

Another environmental factor of potential importance is neurotrophic viral infection, which may induce a protective down-regulation of cellular mRNA translation to prevent viral spread, and viruses in turn have evolved ways to subvert usual cellular RNA responses as an adaptive mechanism[128]. For example, a role for exogenous or endogenous retroviruses in ALS has been suggested[129-131].

Last, there is a long suspicion that ingested or inhaled neurotoxins could predispose to ALS, directly or through epigenetic change[132]. These include potentially excitotoxic or oxidative stressors. Oxidative, mitochondrial, or excitotoxic abnormalities are present in ALS, and are associated with the UPR and generalized stress responses[124].

Disrupted proteostasis and aging and maladaptive repair

Age is by far the most significant risk factor for ALS, with risk increasing with age, although possibly declining somewhat in the elderly[133]. As pointed out many years ago, it is possible that the metabolic demands on a motor neuron increase as the size of the motor unit increases[134], with ever-increasing oxidative stress. Age-related oxidative damage to mitochondria has long been linked to neurodegeneration[135]. As mentioned, oxidative, mitochondrial, and excitotoxic stress are associated with the UPR and generalized stress responses[124]. Also, for uncertain reasons autophagy declines with age[122]. These considerations tie increasing age to perturbed protein homeostasis.

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Some effects might have a more complex relation to age. As peripheral motor neurons malfunction with disease, axons may attempt to regenerate and neighbouring axons sprout into denervated endplate regions. If loss of regenerative potential were paramount, difficulty should increase with age; however over-exuberant axonal regeneration and sprouting could also be harmful under some circumstances but might be seen preferentially in younger adults. Adult mice over-expressing the neuronal growth-associated protein GAP43 have increased death of motor neurons in the spinal cord at the level of sciatic transection[136], and doubly transgenic mice, over-expressing both mSOD1 and GAP43, have accelerated disease onset and terminal decline[137]. GAP43 levels are elevated in the spinal cord of ALS patients[138]. Also, the UPR may lead to cellular de-differentiation[121] and excessive growth signals could induce diseased adult motor neurons to inappropriately re-enter the cell-cycle, resulting in apoptosis[139,140]. Of relevance, the cyclin-dependent kinase Cdk4 is upregulated in mSOD mice[141]. It is presently unclear whether these influences might increase, decline, or remain unchanged with age.

Disrupted proteostasis and inflammation

In most circumstances, cellular stress responses are cytoprotective. From a teleological viewpoint, a survival advantage to the organism might be conferred if this response were relayed to neighbouring cells, perhaps explaining why the UPR is intimately linked to activation of the innate immune system[128]. (For example, sciatic nerve crush activates a UPR in the motor neuron and also elicits an intense ipsilateral regional microglial response in the spinal cord[69]). In this way, the UPR may not be cell autonomous. An organismal response of the first arm of the UPR (XBP-1) has been identified in *C elegans*[142], but it is presently unknown whether this is true in human neurodegenerative disease.

Under certain circumstances, these immune responses may be maladaptive. In classical prion disease, terminal pathology requires microglial involvement[143]. The intense microglial reaction accompanying the symptomatic phases of ALS has been mentioned, as well as the transition from a neuroprotective to neurotoxic phenotype[74]. Given the essential interplay between motor neurons and glia in the pathogenesis of ALS[63], it seems plausible that the death of some motor neurons could activate neighbouring glia and consequently increase the death rate of adjacent and previously-uninvolved motor neurons. Some of the many factors involved in the cross-talk between motor neurons and glia are known[144] but further definition is required.

C. Therapeutic considerations and future challenges

In summary, ALS may be, at its core, a disease of disturbed proteostasis induced with greater likelihood in patients genetically predisposed, potentiated by age, possibly triggered by environmental insult, and worsened terminally by maladaptive inflammation (Figure 2). Protein homeostasis is a tightly-regulated and conserved process that may be difficult to

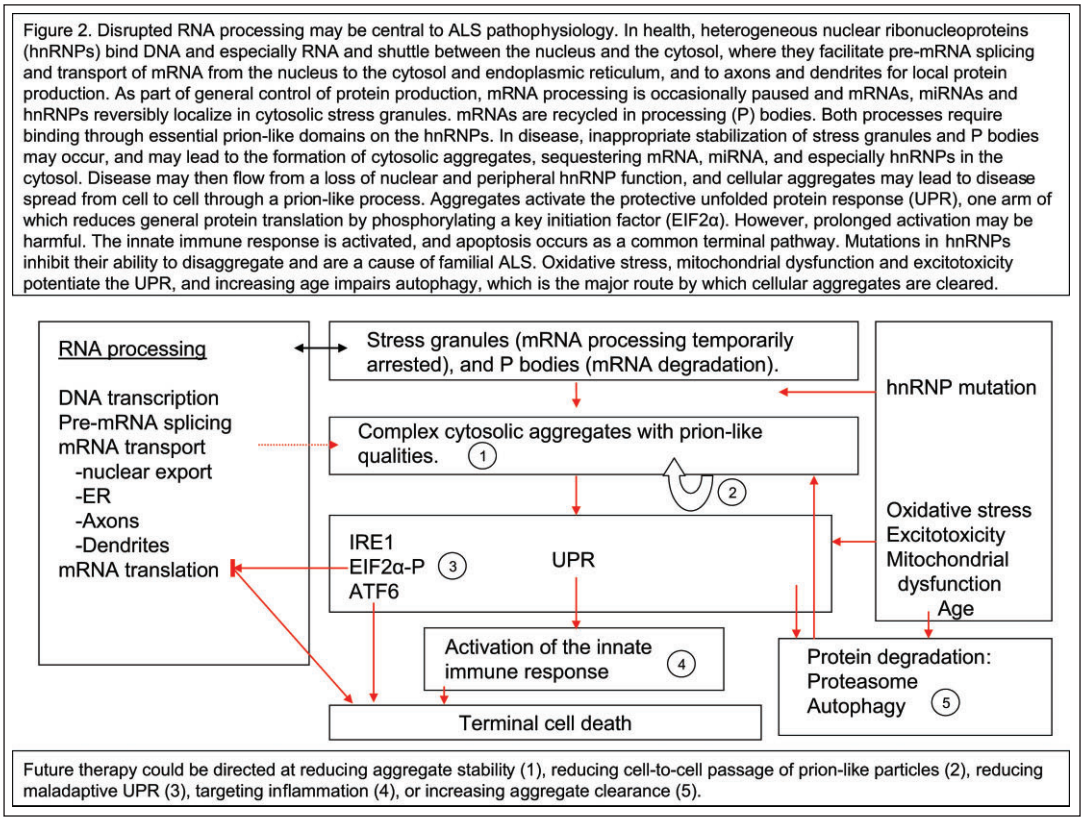

Figure 2. Disrupted RNA processing may be central to ALS pathophysiology. In health, heterogeneous nuclear ribonucleoproteins (hnRNPs) bind DNA and especially RNA and shuttle between the nucleus and the cytosol, where they facilitate pre-mRNA splicing and transport of mRNA from the nucleus to the cytosol and endoplasmic reticulum, and to axons and dendrites for local protein production. As part of general control of protein production, mRNA processing is occasionally paused and mRNAs, miRNAs and hnRNPs reversibly localize in cytosolic stress granules. mRNAs are recycled in processing (P) bodies. Both processes require binding through essential prion-like domains on the hnRNPs. In disease, inappropriate stabilization of stress granules and P bodies may occur, and may lead to the formation of cytosolic aggregates, sequestering mRNA, miRNA, and especially hnRNPs in the cytosol. Disease may then flow from a loss of nuclear and peripheral hnRNP function, and cellular aggregates may lead to disease spread from cell to cell through a prion-like process. Aggregates activate the protective unfolded protein response (UPR), one arm of which reduces general protein translation by phosphorylating a key initiation factor (EIF2α). However, prolonged activation may be harmful. The innate immune response is activated, and apoptosis occurs as a common terminal pathway. Mutations in hnRNPs inhibit their ability to disaggregate and are a cause of familial ALS. Oxidative stress, mitochondrial dysfunction and excitotoxicity potentiate the UPR, and increasing age impairs autophagy, which is the major route by which cellular aggregates are cleared.

Future therapy could be directed at reducing aggregate stability (1), reducing cell-to-cell passage of prion-like particles (2), reducing maladaptive UPR (3), targeting inflammation (4), or increasing aggregate clearance (5).

Figure 2: *Disrupted RNA processing may be central to ALS pathophysiology.*

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therapeutically perturb. The focal nature of ALS complicates therapeutic considerations, as exposing all cells to a therapy for a vital function but directed at a small number of motor neurons will almost certainly fail.

mSOD mice haploinsufficient for PERK (reducing Eif2α-P) have accelerated disease course[145], and blocking Eif2α-P dephosphorylation in mSOD mice modestly prolongs disease onset[69]. This suggests that reducing protein translation and unloading the ER may be helpful, and has raised the possibility of therapeutic intervention by interfering with Eif2α-P dephosphorylation.

Even ignoring the focal nature of ALS, this approach is unlikely to prove satisfactory. If a cell is subjected to prolonged stress and phosphorylation of Eif2α is maintained, protein production is reduced, possibly accompanied by DNA methylation and reduced transcription[146], cell viability is compromised, and the third arm of the UPR initiates apoptosis. Sustained activation of Eif2α-P has been shown to contribute to neuronal death in classical prion disease[147], and sustained activation of Eif2α-P may be occurring in ALS as Eif2α-P levels are elevated in the spinal cord of ALS patients[124]. Indeed this irreversible phase may be a common terminal event in ALS motor neurons, further strengthening the conceptual link with classical prion disease.

Nonetheless, some aspects of disrupted proteostasis might be amenable to intervention, especially with better understanding of those triggers leading to an irreversible UPR and apoptosis. It will be important to elucidate the mechanisms underlying the above-mentioned sharp reduction in proteasomal function and concomitant induction of the UPR preceding motor neuron death[69], as there may be elements[122] that do not implicate usual physiological proteostasis (and therefore might be more amenable to intervention). Destabilization of toxic aggregates would be a promising approach. Treatments aimed at inducing heat shock proteins, and facilitating autophagy, might be helpful in reducing the progression of prion-like disease[148]. Therapeutic drainage of CSF could reduce the intercellular passage of prion-like proteins. This has failed in Alzheimer's disease[149], but could possibly show greater success in lower limb onset ALS.

Reducing the damage from deleterious inflammation remains a promising approach. Non-specific anti-inflammatory treatment has been unhelpful, but treatment might be more successful when targeting specifically factors linking the UPR to activation of the innate immune system (as correcting these might perturb normal cells to a lesser degree). Likewise, treatment of ALS-associated metabolic abnormalities harmful to motor neurons has been futile, but concomitant treatment might be beneficial once disturbed proteostasis is addressed. In dominant FALS, inactivation of the mutated allele could help stabilize the UPR and slow progression to an apoptotically-defined response.

In this context, attention must again be turned to the appropriateness of the mSOD mouse as a disease model, as it is likely that protein half-lives in pathological aggregates are similarly long in man and mouse, yet the clinical disease process in the mouse takes a little more than a month to run its course from start to finish. There may simply not be enough time for therapies to work in the mouse, especially if distal axonal mRNA translation is important.

Conclusions

There are plausible reasons to believe that ALS treatments to date have failed because we have targeted coincident or predisposing processes rather than those more fundamental to the progressive dysfunction and death of motor neurons. Processes central to motor neuron death may implicate aberrant proteostasis, involving cytosolic sequestration of mRNA, miRNA and RNA binding proteins, possible cell-to-cell spread of self-templating toxic aggregates, sustained UPR and terminally, a non-reversible transition to an apoptotically-defined response. Once activated by whatever means, these mechanisms may be at least partially self-perpetrating and less dependent on the underlying trigger, explaining a common disease phenotype in sporadic disease and in all forms of FALS in spite of a variety of predisposing causes.

Depending on one's preference in disease classification, the ALS disease process (as distinct from the underlying predisposing condition) may best be considered distinct and in most patients relatively short.

Protein homeostasis is vital and conserved and interventions may be difficult. Nonetheless, at least some elements may be amenable to intervention. In unfavourable circumstances, we might turn a short bad disease into a long bad disease, while in favourable circumstances with better understanding and multiple interventions some forms of ALS may be treatable or even reversible. Early detection and early treatment would seem essential.

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