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Critical Review

Role of Androgen Receptor PolyQ Chain Elongation in Kennedy's Disease and Use of Natural Osmolytes as Potential Therapeutic Targets

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Summary

Instability of CAG triplet repeat encoding polyglutamine (polyQ) stretches in the gene for target protein has been implicated as a putative mechanism in several inherited neurodegenerative diseases. Expansion of polyQ chain length in the androgen receptor (AR) causes spinal and bulbar muscular atrophy (SBMA) or Kennedy's disease. Although the mechanisms underlying gain-of-neurotoxic function are not completely understood, suggested pathological mechanisms of SBMA involve the formation of AR nuclear and cytoplasmic aggregates, a characteristic feature of patients with SBMA. The fact that certain AR coactivators are sequestered into the nuclear inclusions in SBMA possibly through protein–protein interactions supports the notion that AR transcriptional dysregulation may be a potential pathological mechanism leading to SBMA. AR conformational states associated with aberrant polyQ tract also modulate the interaction of AR with several coactivators. In many cases, such diseases can be treated through protein replacement therapy; however, because recombinant proteins do not cross the blood–brain barrier, the effectiveness of such therapies is limited in case of neurodegenerative diseases that warrant alternative therapeutic approaches. Among different approaches, inhibiting protein aggregation with small molecules that can stimulate protein folding and reverse aggregation are the most promising ones. Thus, naturally occurring osmolytes or "chemical chaperones" that can easily cross the blood–brain barrier and stabilize the functional form of a mutated protein by shifting the folding equilibrium away from degradation and/or aggregation is a useful therapeutic approach. In this review, we discuss the role of polyQ chain length extension in the pathophysiology of SBMA and the use of osmolytes as potential therapeutic tool.
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Keywords protein folding; aggregation; neurodegenerative diseases; CAG codon; spinal and bulbar muscular atrophy.

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INTRODUCTION

Protein folding/unfolding mainly depends on the intrinsic properties of the sequence of amino acids and cellular milieu and is a crucial way of regulating biological activities and to carry out essential cellular functions (*1, 2*). Functionally relevant proteins that escape the cellular quality-control mechanisms involved in the process of regulating protein folding can lead to pathological conditions (*3*). This phenomenon is quite common in several neurodegenerative diseases in which protein misfolding converts them to insoluble fibrils or amyloidogenic plaques (*4, 5*). In many cases, this appears to be due to mutations in the gene of the associated protein; for example, instability of CAG triplet repeat encoding polyglutamine (polyQ) stretches in the gene for the target protein has been implicated as a putative mechanism in several inherited neurodegenerative diseases (*6, 7*). Although the target proteins associated with these phenotypes are quite distinct, the mutated forms share an expanded CAG gene product as a mediator of aggregation (*8, 9*). Transgenic mice studies reveal that androgen receptor (AR) gene with expanded CAG codon repeats exhibits selective neurological phenotypes and neurodegeneration and causes spinal and bulbar muscular atrophy (SBMA) or Kennedy's disease (an X-linked progressive motor neuron disease), without affecting other neurons (*10, 11*). The suggested pathological mechanisms involve the formation of nuclear and cytoplasmic aggregates, a characteristic feature of patients with these diseases (*12, 13*).

There are suggestions that newly synthesized peptide (highly disordered) passes through a transitional phase (partially folded) state before adopting a fully folded functionally active form *in vivo* (Fig. 1) (*14*). However, under certain conditions, during the process of folding, protein unfolds, at least partially, and becomes prone to aggregation (*14*). This can result in the formation of highly organized fibrils and plaques that can give rise to pathological conditions leading to diseases (*15*). Thus, to avoid aggregation in the biological environment, the ensembles of unfolded or partially folded protein population (at a given

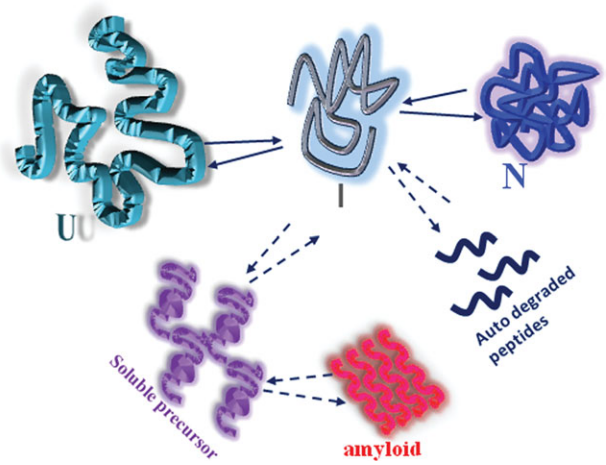

Figure 1. Schematic diagram of some of the states accessible to a polypeptide chain following its biosynthesis (*14*). A newly synthesized peptide (U, unfolded state) goes through an intermediate phase (I, partially structured) state before adopting a fully folded functionally active form (N, globular native form). The intermediate, I, conformation is prone to degradation/aggregation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

time) must be in a small proportion. In this regard, the cooperative nature of two-state protein folding process could be crucial for avoiding protein/peptide aggregation (*16*). For such purposes, nature has developed the strategy of using specific solutes, such as "chemical chaperones" or organic osmolytes, which are widely present in living cells/tissues, and can have powerful effects on protein folding and stability (*17*). These compounds exert a force to cooperatively fold proteins (that are highly unstructured and/or susceptible to aggregation/misfolding) into native-like, functional species without challenging the rules of protein folding (*17, 18*). Thus, naturally occurring osmolytes can be used as potential therapeutic targets for such diseases, which have certain added therapeutic advantages: (i) no fear of any major side effects due to their uses and (ii) can easily cross blood–brain barriers, which is a major drawback in using recombinant proteins as therapeutic tools for neurodegenerative diseases. In this review article, we discuss the role of AR polymorphism in Kennedy's disease and the use of natural osmolytes as potential therapeutic strategies.

POSSIBLE MECHANISMS ASSOCIATED WITH SBMA PATHOPHYSIOLOGY

The pathophysiology and the underlying mechanisms of neuronal cell death and neurotoxicity in polyQ diseases is a complex phenomenon. In recent years, works from several laboratories have reported that many pathways such as direct activation of cell death signaling, mitochondrial abnormalities, transcriptional dysregulation, proteasome impairment, defects in axonal transport, metabolic stress, accumulation of free radicals, and/or protein misfolding/unfolding can lead to the polyQ chain length-mediated cell death (*19*). It has been reported that ubiquitin-proteasome pathway functions during cytoplasmic aggregation in SBMA as suggested by accumulation of a proteasome reporter in the absence of aggregates in motor neurons expressing AR polyQ (*19*). Furthermore, induction of AR polyQ aggregation allowed the normal clearance of this proteasome reporter, suggesting that aggregation contributed to proteasome desaturation, an effect not related to AR nuclear translocation (*19*). There are studies also showing that increased autophagy could clear the mutated AR protein and thereby reducing polyQ-mediated neurotoxicity (*20*). One of the first therapeutic approaches in polyQ diseases has been aimed at the prevention of aggregation using small molecules and/or cellular levels of molecular chaperones, which could prevent the formation of polyQ aggregates.

ANDROGEN RECEPTOR AND POLYMORPHISM INVOLVING THE EXPANSION OF CAG CODON REPEAT

Androgens are involved in the development and functional maturation of several tissues, including skeletal muscle, bone marrow, hair follicles, male reproductive system, and brain (*21*). Most of the biological effects of the androgens are medi-

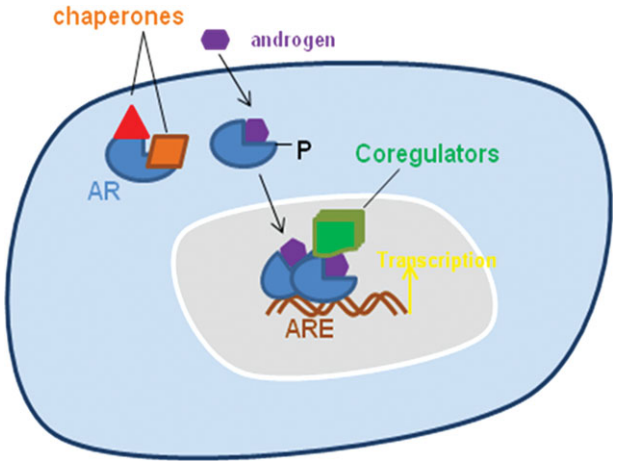

Figure 2. A classical model of the AR action. Unliganded AR resides in the cytosol associated with several heat-shock proteins and other chaperone proteins. Androgen steroid/hormone penetrates the cell membrane and binds to the receptor. Thus, the ligand displaces associated proteins, and the receptor undergoes post-translational modifications, for example, site-specific phosphorylation (P) and conformational rearrangements, which allows the AR to enter the nucleus and bind to its site-specific DNA sequences termed as androgen response element (ARE). At this time, the receptor interacts with various coregulatory proteins that mediate cross talks with the basal transcription machinery complex and chromatin remodeling leading to the transcriptional regulation of the target gene. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

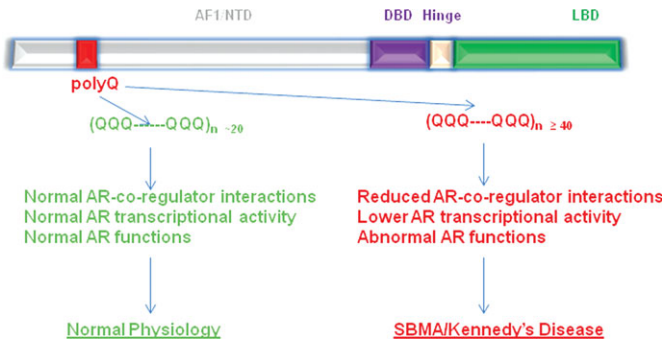

Figure 3. A diagram showing possible physiological and pathological scenarios due to altered polyQ chain length in the AR (*25*). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ated through the AR, which passes signals from an androgen to the target genes by interacting with specific response-element DNA sequences and various coregulatory proteins (Fig. 2). The precise nature and actions of these coregulatory protein interactions appear to be cell/tissue specific and promoter specific (*22*). Because of the critical role of these coregulators in the AR-mediated gene regulation, any dysregulation of AR:coregulator interactions may perturb normal physiology leading to disease conditions (*22*). This has been supported by the facts that critical AR interacting coregulators (steroid receptor coactivator-1 [SRC-1] and β-catenin) have been reported as prognosis or diagnostic markers in disease development and progressions (*23*). In addition, several types of mutations in the AR gene have been linked to endocrine dysfunctions (*24*). Among the AR mutations is the polymorphism involving the expansion of CAG codon repeat, coding for a polyQ tract in its N-terminal domain (NTD), which possesses a powerful transactivation function (AF1) region (Fig. 3) (*25*).

The molecular mechanisms of these alterations are not fully understood but are suggested to involve modulation of AR transcriptional potency, which inversely correlates with the polyQ chain length (*26, 27*). The exact mechanisms of how CAG polymorphism alters efficacy and potency of transactivation are unknown. Although ligand-specific multiscale modeling to quantify nonlinear contributions of AR transcriptional potency and efficacy has been explored (*28, 29*), the effects mediated by polyQ perturbations in the NTD are mostly ligand independent. Thus, a comprehensive model of AR transcriptional potency and efficacy that can allow the signal amplification and aberrant regulation caused by alteration in polyQ repeat length must include ligand and ligand binding domain mutation-independent signaling node. Studies in human tissues and cell culture have suggested that the toxic form of AR is a short NTD fragment containing the expanded polyQ chain, which forms cytoplasmic and nuclear aggregates (*30*). Because of the importance of interaction of p160 group of proteins (which consists of SRC-1, -2, and -3) with AR AF1/NTD in the regulation of its transcriptional potency and efficacy and the fact that increasing polyQ chain length inhibits this critical protein–protein interaction, it is hypothesized that this compromised situation with increased polyQ chain may be a potential pathological mechanism leading to AR-related hereditary diseases.

TRANSCRIPTIONAL DYSREGULATION OF THE AR IN SBMA OR KENNEDY'S DISEASE

SBMA is one of the first neurodegenerative diseases characterized by the death of motor neurons mainly located in the spinal cord and bulbar regions for which the molecular basis was discovered to be neurotoxicity caused by the expansion of a polyQ repeat (usually ranging from 40 to 62 when compared with $\sim$ 20 in normal adults) in the highly expressed causative gene, AR (*31*). Using drosophila model, it was recently reported that initiation of SBMA pathogenesis is closely related to the formation of androgen-dependent intracellular AR aggregates (*32*) due to either homodimerization or production of aberrant conformational changes in the AR protein (*33, 34*). Extended polyQ chain length induces the formation of inclusion bodies in the affected neurons, suggesting that proteolytic cleavage may be responsible for enhanced toxicity of the AR gene products (*31*). The fact that certain AR coactivators are sequestered into the nuclear inclusions in SBMA possibly through protein–protein interactions (*30*) supports the notion that the AR transcriptional dysregulation may be a potential pathological mechanism leading to SBMA (*30*). Like the activation domain of many transcription factors, AR NTD/AF1 exists in an intrinsically disordered conformation, and under physiological conditions, NTD/AF1 undergoes disorder/order transition via intermolecular and intramolecular communications, which allows AR's AF1/NTD surfaces to interact with specific coregulatory proteins, critical for the final outcome of target gene expression regulated by AR (*35*).

The conformational states associated with aberrant polyQ tract inversely modulate the interaction of AR NTD/AF1 with several coactivators, including SRC-1a, SRC-3 (AIB1), and SRC-2/GRIP1 (*26, 35*). However, quantitative assessment of contribution of each of these factors in patients with SBMA is required to assess abrogation of AR signaling *in vivo* before targeting their therapeutic purposes. Based on the previous published data (*35, 36*), it is believed that osmolyte-induced conformation in NTD/AF1 should allow AF1's efficient interactions with SRCs and thereby AR's transcriptional activity in a cell- and promoter-specific manner. However, this needs to be tested whether the AR NTD even with extended polyQ chain length is capable of mediating AF1 activity via folding and binding different coregulatory proteins, including SRCs. As a first step toward this goal, future research should be aimed at mapping the protein interaction surfaces between SRCs and the AR NTD/AF1 and at examining the effects of SRCs on conformational dynamics of NTD/AF1 with varying polyQ chain lengths. Given the findings that osmolyte-induced conformation of the AR NTD/AF1 facilitates its interactions with coregulatory proteins including SRCs, it is logical to propose that osmo-

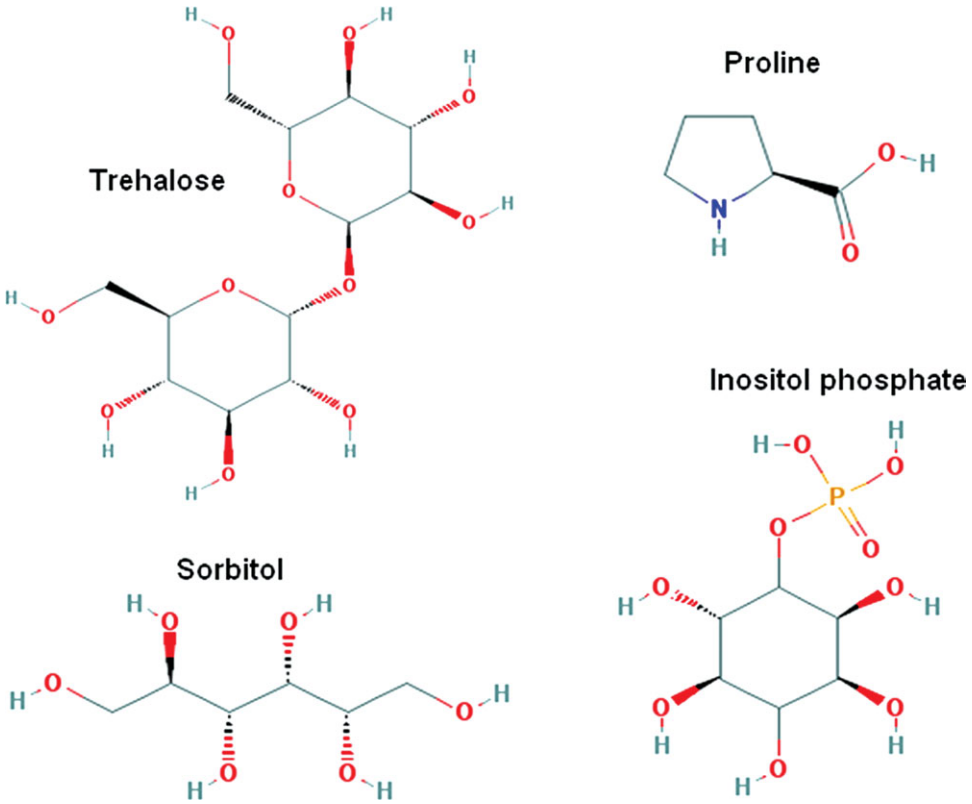

Figure 4. Structure of some well-known naturally occurring osmolytes (source: PubChem structure search). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

lytes can be used to stabilize the AR NTD structure even with extended polyQ chain length such that in this conformation, AF1/NTD's interaction with other coregulatory proteins is restored, which results into normal AR functions and thereby preventing elongated polyQ chain aggregation.

NATURALLY OCCURRING OSMOLYTES: A POTENTIAL THERAPEUTIC TOOL FOR SBMA AND POSSIBLY OTHER NEURODEGENERATIVE DISEASES

Alterations in normal cellular conditions can make most proteins susceptible to misfolding and/or aggregation, resulting into a partial or complete loss of their activity (*37*). To counterbalance any such eventuality, cells fine tune itself by accumulating high concentrations of small organic molecules (osmolytes), often as a result of an adaptive de novo synthesis (*38*). A list of some potent osmolytes used by nature is shown in Fig. 4. Because of their flexible structures, intrinsically disordered regions/domains of many signaling proteins including AR NTD/AF1 are capable of creating favorable surfaces for their efficient interactions with their target proteins (*39*) and can also be induced to fold spontaneously to native, functional forms by addition of certain osmolytes to the solvent (*40, 41*). In this osmolyte-induced conformation, NTD/AF1 of the AR binds strongly to SRC-1 (*41*). These findings could have far-reaching implications in regulating/maintaining the AR's natural folding and transcriptional activity. It is important to note that certain osmolytes can be used to protect proteins from misfolding/aggregation and thereby maintaining/restoring their intracellular functional activity (*42, 43*), which otherwise may be prone to certain disease conditions. In fact, the utility of osmolytes as potential therapeutic interventions for neurodegenerative diseases, particularly associated with pathological conditions arising due to polyQ chain length extension, has been supported by studies in which osmolyte trehalose has been used as a preventive tool for Huntington's disease in transgenic animal mice (*44*). Osmolyte trehalose has been reported to activate macroautophagy possibly by the stabilization of the expanded polyQ protein and thereby preventing conversion of the expanded polyQ into aggregation-prone β-sheet-rich conformation (*45, 46*).

Either by inefficient folding/excessive degradation or by excessive forced folding/aggregation, several misfolding-prone proteins are known to result into deleterious genetic disorders, which are often treated through protein replacement therapy. However, the effectiveness of such protein replacement therapies is limited in case of neurodegenerative diseases such as SBMA. This is because recombinant proteins fail to efficiently cross the blood–brain barrier. Therefore, the mainstay of research efforts must be focused on further characterizing the mechanisms of polyQ chain length extension in mediating neurodegenerative diseases and developing new therapeutic strategies to fight them with a better benefit-to-risk-ratio. Or-

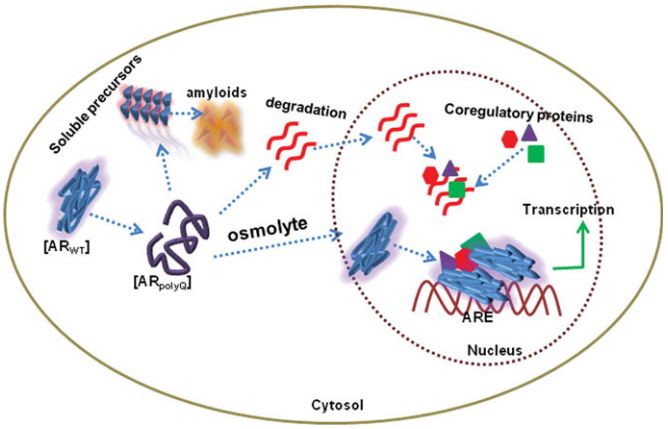

Figure 5. A possible therapeutic mechanism of use of osmolytes to protect misfolded/aggregation-prone AR in SBMA (*47*). The expanded AR polyQ tract allows transition into a distinct conformation that may cause toxicity as a monomer or it may self-associate to form toxic oligomers, which could assemble into larger aggregates leading to intracellular inclusions. The principal toxic effects of the aberrantly folded protein may include alterations in transcription, metabolism, or impairment of the proteasome or stress response pathways. Under pathological conditions (due to polyQ chain length extension), the structure of natively folded functional WT AR protein (AR_{WT}) is compromised due to partial folding (AR_{polyQ}) that could either result in unwanted degradation or into formation of soluble precursors that forms amyloids. These polyQ-containing peptide fragments enter the nucleus, sequester AR coregulatory proteins resulting into the formation of intranuclear inclusions and thereby blocking the AR-mediated transcriptional regulation. Osmolytes may convert (AR_{polyQ}) back into (AR_{WT}) conformation, and thereby restoring proper functions of protein by allowing it to translocate to nucleus where it binds to androgen response element (ARE) DNA sequences and interacts with critical coregulatory proteins (shown by different colors and shapes) leading to the AR target gene transcription. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ganic osmolytes that can easily cross blood–brain barrier and stabilize functionally active conformation of a mutated protein, which may otherwise have become susceptible to degradation and/or aggregation, appear to be a useful therapeutic approach for such diseases. The use of naturally occurring osmolytes is an innovative idea because of no major risk of side effects, which is a critical factor in designing a drug target for neurodegenerative diseases commonly associated with elder population. In this period of emphasis on the relevance of results of basic research to practical uses, better understanding of the osmolyte-induced functional folding of the AR and its polyQ mutants will be of great clinical value for SBMA.

The essence of the therapeutic model (Fig. 5) proposed here is that under pathological conditions (due to polyQ chain length extension), the natively folded functional structure of the AR protein is compromised that could either result in unwanted degradation or into formation of soluble precursors, which forms amyloids. Osmolytes can directly convert (AR_{polyQ}) back into (AR_{WT}) conformation, and thereby restoring proper functions of AR protein. For function, the AR NTD/AF1 even with an extended polyQ chain should have a stable functional structure capable of interacting specifically with critical coactivators/corepressors and with one or more proteins of the basal transcription initiation complex. This interaction in turn should help to prevent the aggregation mediated by polyQ chain elongation.

SUMMARY AND FUTURE PERSPECTIVES

In recent years, outstanding progress has been made in the development of therapeutic strategies targeting diseases associated with protein misfolding/aggregation. Among different approaches, inhibiting protein aggregation with small molecules that can stimulate protein folding and reverse aggregation are the most promising ones. Thus, naturally occurring osmolytes can provide a potent therapeutic approach for the prevention of protein aggregation due to elongated polyQ chain length, the cause of several neurodegenerative diseases including SBMA for which no cure is available. Our long-term aim should be to expand *in vitro* findings into mouse models of SBMA that can provide a platform for translational research for the development of clinical uses of these natural small molecules. Mutant polyQ-expanded AR protein is ubiquitously expressed but leads to selective lower motoneuron loss in the spinal cord and bulbar nuclei of the brainstem. Although the cause of the motoneuron death and dysfunction in SBMA, which is restricted to particular lower and bulbar motoneurons, is largely unknown, current evidence supports the transcriptional dysregulation by mutant AR in a mouse model of SBMA (*48*), prompting the need for the development of therapeutic strategies for SBMA based on these targets. An understanding of the proper mechanism of action of osmolytes will provide important clues about their efficacy and should have far-reaching consequences in developing better therapeutic tools for the prevention and/or management of neurodegenerative diseases such as SBMA.

REFERENCES

1. Bahar, I., Lezon, T. R., Yang, L. W., and Eyal, E. (2010) Global dynamics of proteins: bridging between structure and function. *Annu. Rev. Biophys.* **39**, 23–42.
2. Hardesty, B., Tsalkova, T., and Kramer, G. (1999) Co-translational folding. *Curr. Opin. Struct. Biol.* **9**, 111–114.
3. Kubota, H. (2009) Quality control against misfolded proteins in the cytosol: a network for cell survival. *J. Biochem.* **146**, 609–616.
4. Naiki, H. and Nagai, Y. (2009) Molecular pathogenesis of protein misfolding diseases: pathological molecular environments versus quality control systems against misfolded proteins. *J. Biochem.* **146**, 751–756.
5. Chiti, F. and Dobson, C. M. (2006) Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* **75**, 333–366.
6. Bauer, P. O. and Nukina, N. (2009) The pathogenic mechanisms of polyglutamine diseases and current therapeutic strategies. *J. Neurochem.* **110**, 1737–1765.

7. Muchowski, P. J. and Wacker, J. L. (2005) Modulation of neurodegeneration by molecular chaperones. *Nat. Rev. Neurosci.* **6**, 11–22.
8. Bates, G. P., Mangiarinim L., and Daviesm S. W. (1998) Transgenic mice in the study of polyglutamine repeat expansion diseases. *Brain Pathol.* **8**, 699–714.
9. Smith, D. L., Portier, R., Woodman, B., Hockly, E., Mahal, A., et al. (2001) Inhibition of polyglutamine aggregation in R6/2 HD brain slices-complex dose-response profiles. *Neurobiol. Dis.* **8**, 1017–1026.
10. Cowin, R. M., Roscic, A., Bui, N., Graham, D., Paganetti, P., et al. (2012) Neuronal aggregates are associated with phenotypic onset in the R6/2 Huntington's disease transgenic mouse. *Behav. Brain Res.* **229**, 308–319.
11. Simanainen, U., Brogley, M., Gao, Y. R., Jimenez, M., Harwood, D. T., et al. (2011) Length of the human androgen receptor glutamine tract determines androgen sensitivity in vivo. *Mol. Cell. Endocrinol.* **342**, 81–86.
12. Tang, B., Seredenina, T., Coppola, G., Kuhn, A., Geschwind, D. H., et al. (2011) Gene expression profiling of R6/2 transgenic mice with different CAG repeat lengths reveals genes associated with disease onset and progression in Huntington's disease. *Neurobiol. Dis.* **42**, 459–467.
13. Panet-Raymond, V., Gottlieb, B., Beitel, L. K., Schipper, H., Timiansky, M., et al. (2001) Characterization of intracellular aggregates using fluorescently-tagged polyglutamine-expanded androgen receptor. *Neurotox. Res.* **3**, 259–275.
14. Dobson, C. M. (1999) Protein misfolding, evolution and disease. *Trends Biochem. Sci.* **20**, 329–332.
15. Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., et al. (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature* **416**, 507–511.
16. Dobson, C. M. and Karplus, M. (1999) The fundamentals of protein folding: bringing together theory and experiment. *Curr. Opin. Struct. Biol.* **9**, 92–101.
17. Burg, M. D. (1995) Molecular basis of osmotic regulation. *Am. J. Physiol.* **268**, F983–F996.
18. Qu, Y., Bolen, C. L., and Bolen, D. W. (1998) Osmolyte-driven contraction of a random coil protein. *Proc. Natl. Acad. Sci. USA* **95**, 9268–9273.
19. Bauer, P. O. and Nukina, N. (2009) The pathogenic mechanisms of polyglutamine diseases and current therapeutic strategies. *J. Neurochem.* **110**, 1737–1765.
20. Bauer, P. O., Wong, H. K., Oyama, F., Goswami, A., Okuno, M., et al. (2009) Inhibition of rho kinases enhances the degradation of mutant huntingtin. *J. Biol. Chem.* **284**, 13153–13164.
21. Bagatell, C. J. and Bremner, W. J. (1996) Androgens in men—uses and abuses. *N. Engl. J. Med.* **334**, 707–714.
22. Heinlein, C. A. and Chang, C. (2002) Androgen receptor (AR) coregulators: an overview. *Endocr. Rev.* **23**, 175–200.
23. Masiello, D., Chen, S. Y., Xu, Y., Verhoeven, M. C., Choi, E., et al. (2004) Recruitment of β-catenin by wild-type or mutant androgen receptors correlates with ligand-stimulated growth of prostate cancer cells. *Mol. Endocrinol.* **18**, 2388–2401.
24. Poletti, A., Negri-Cesi, P., and Martini, L. (2005) Reflections on the diseases linked to mutations of the androgen receptor. *Endocr. Rev.* **28**, 243–262.
25. Kumar, R., Atamna, H., Zakharov, M. N., Bhasin, S., Khan, S. H., et al. (2011) Role of the androgen receptor CAG repeat polymorphism in prostate cancer, and spinal and bulbar muscular atrophy. *Life Sci.* **88**, 565–571.
26. Irvine, R. A., Ma, H., Yu, M. C., Ross, R. K., Stallcup, M. R., et al. (2000) Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. *Hum. Mol. Genet.* **9**, 267–274.
27. Tut, T. G., Ghadessy, F. J., Trifiro, M. A., Pinsky, L., and Yong, E. L. (1997) Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. *J. Clin. Endocrinol. Metab.* **82**, 3777–3782.
28. Mhatre, A. N., Trifiro, M. A., Kaufman, M., Kazemi-Esfarjani, P., Figlewicz, D., et al. (1993) Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. *Nat. Genet.* **5**, 184–188.
29. Stanford, J. L., Just, J. J., Gibbs, M., Wicklund, K. G., Neal, C. L., et al. (1997) Polymorphic repeats in the androgen receptor gene: molecular markers of prostate cancer risk. *Cancer Res.* **57**, 1194–1198.
30. Diamond, M. I., Robinson, M. R., and Yamamoto, K. R. (2000) Regulation of expanded polyglutamine protein aggregation and nuclear localization by the glucocorticoid receptor. *Proc. Natl. Acad. Sci. USA* **97**, 657–661.
31. Palazzolo, I., Gliozzi, A., Rusmini, P., Sau, D., Crippa, V., et al. (2008) The role of the polyglutamine tract in androgen receptor. *J. Steroid Biochem. Mol. Biol.* **108**, 245–253.
32. Nedelsky, N. B., Pennuto, M., Smith, R. B., Palazzolo, I., Moore, J., et al. (2010) Native functions of the androgen receptor are essential to pathogenesis in a Drosophila model of spinobulbar muscular atrophy. *Neuron* **67**, 936–952.
33. Rusmini, P., Sau, D., Crippa, V., Palazzolo, I., Simonini, F., et al. (2007) Aggregation and proteasome: the case of elongated polyglutamine aggregation in spinal and bulbar muscular atrophy. *Neurobiol. Aging* **28**, 1099–1111.
34. Perutz, M. F., Johnson, T., Suzuki, M., and Finch, J. T. (1994) Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proc. Natl. Acad. Sci. USA* **91**, 5355–5358.
35. Reid, J., Betney, R., Watt, K., and McEwan, I. J. (2003) The androgen receptor transactivation domain: the interplay between protein conformation and protein–protein interactions. *Biochem. Soc. Trans.* **31**, 1042–1046.
36. Kumar, R. (2008) Osmolyte-induced folding of an intrinsically disordered region of glucocorticoid receptor. *J. Recept. Signal Transduct. Res.* **28**, 465–474.
37. Závodszky, P., Kardos, J., Svingor, A., and Petsko, G. A. (1998) Adjustment of conformational flexibility is a key event in the thermal adaptation of proteins. *Proc. Natl. Acad. Sci. USA* **95**, 7406–7411.
38. Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D., and Somero, G. N. (1982) Living with water stress: evolution of osmolyte systems. *Science* **217**, 1214–1222.
39. Kumar, R. and McEwan, I. J. (2012) Allosteric modulators of steroid hormone receptors: structural dynamics and gene regulation. *Endocr. Rev.* **33**, 271–299.
40. Kumar, R., Lee, J. C., Bolen, D. W., and Thompson, E. B. (2001) The osmolyte-induced conformation of the glucocorticoid receptor AF1/tau1 domain binds coregulators. *J. Biol. Chem.* **276**, 18146–18152.
41. Kumar, R., Betney, R., Li, J., Thompson, E. B., and McEwan, I. J. (2004) Induced α-helix structure in AF1 of the androgen receptor upon binding transcription factor TFIIF. *Biochemistry* **43**, 3008–3013.
42. Soto, C. (2001) Protein misfolding and disease: protein refolding and therapy. *FEBS Lett.* **498**, 204–207.
43. Powers, E. T., Morimoto, R. I., Dillin, A., Kelly, J. W., and Balch, W. E. (2009) Biological and chemical approaches to diseases of proteostasis deficiency. *Ann. Rev. Biochem.* **78**, 959–991.
44. Tanaka, M., Machida, Y., Niu, S., Ikeda, T., Jana, N. R., et al. (2004) Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nat. Med.* **10**, 148–154.
45. Nagai, Y., Fujikake, N., Ohno, K., Higashiyama, H., Popiel, H. A., et al. (2003) Prevention of polyglutamine oligomerization and neurodegeneration by the peptide inhibitor QBP1 in Drosophila. *Hum. Mol. Genet.* **12**, 1253–1259.
46. Nagai, Y., Inui, T., Popiel, H. A., Fujikake, N., Hasegawa, K., et al. (2007) A toxic monomeric conformer of the polyglutamine protein. *Nat. Struct. Mol. Biol.* **14**, 332–340.
47. Tanaka, M., Machida, Y., and Nukina, N. (2005) A novel therapeutic strategy for polyglutamine diseases by stabilizing aggregation-prone proteins with small molecules. *J. Mol. Med.* **83**, 343–352.
48. McManamny, P., Chy, H. S., Finkelstein, D. I., Craythorn, R. G., Crack, P. J., et al. (2002) A mouse model of spinal and bulbar muscular atrophy. *Hum. Mol. Genet.* **11**, 2103–2111.