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Mini-Review

SCA7: Is the rub in the DUB?

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ABSTRACT

Spinocerebellar Ataxia type 7 (SCA7) is one of 9 neurodegenerative diseases caused by polyglutamine (polyQ) expansions in target proteins. The length of the polyQ expansions are inversely correlated with the age of onset and directly correlated with the severity of the disease. Although the affected protein is different in each of the polyQ diseases, common cellular and molecular changes are associated with the expansions, including abnormal gene expression patterns, the presence of nuclear inclusions, and altered ubiquitin levels. The mechanistic basis for these changes, or how these features give rise to progressive ataxias, is not clear. Clues to these questions may come from studies of Atxn7, the protein affected in SCA7. Atxn7 is part of the SAGA complex, which houses both histone acetyltransferase (HAT) and deubiquitinase (DUB) activity. Both of these activities are essential for the role of SAGA in gene regulation, and disruption of these functions by polyQ-Atxn7 might directly contribute to the disease phenotype. Recent studies indicate that the SAGA DUB module, which includes Atxn7, has nonhistone substrates, raising the possibility that loss or misdirection of the DUB module might contribute to the abnormal accumulation of ubiquitin observed in SCA7 cells. Here we explore this idea and present a model for how polyQ-Atxn7 might act in a dominant manner over time to disrupt SAGA functions, disturbing both transcriptional and non-transcriptional processes, ultimately leading to development of SCA7.

KEYWORDS: SCA7, ubiquitin, SAGA, deubiquitinase, neurodegenerative disease

INTRODUCTION

Even though genetic defects associated with many inherited neurodegenerative diseases have been identified, the molecular mechanisms underlying the onset or progression of these diseases have yet to be clearly defined. PolyQ expansion diseases typified by SCA7 affect a single genomic locus that is often expressed ubiquitously, but which selectively affects particular neuronal cell subtypes, leading to distinct neurological symptoms [1-4]. Moreover, cells expressing the polyQ protein also express wild type protein from the non-expanded allele, indicating the polyQ protein acts in a dominant fashion to cause the disease state. In both SCA7 and SCA1, the affected protein is part of a larger complex, and depending on the context of the other proteins in the complex, the polyQ expansion can cause gain- or loss-of-function [5-7].

Atxn7, and its polyQ derivative, are part of a multisubunit histone acetyltransferase (HAT) complex termed SAGA [5]. Although best characterized in terms of its HAT catalytic subunit, Gcn5, SAGA contains a second enzymatic module that houses deubiquitinating (DUB) activity [8]. Atxn7 is an important subunit of the DUB module, but it is not yet known whether or how polyQ-Atxn7 affects this function [8]. Interestingly, accumulation of ubiquitinated proteins is observed

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in SCA7 and other polyQ disease states [9-15]. Here, we review SAGA functions and suggest a model for how loss of the DUB module might upset ubiquitin homeostasis and contribute to neurodegeneration.

The ubiquitin proteasome system in polyQ diseases

As in other cell types, neurons utilize the ubiquitin-proteasome system for protein turnover in controlling synaptic activity and neurite projection [16, 17]. Recent findings further indicate that neurons maintain higher levels of free ubiquitin than do other cell types [18], and defects in the ubiquitin-proteasome system have been suggested to contribute to neural dysfunction [19-23]. In polyQ diseases, both the polyQ-protein and ubiquitin accumulate and are often sequestered into nuclear inclusions [11, 13, 15]. Although these accumulations might reflect reduced activity of the proteasome, this does not appear to be the case in SCA7, as normal proteasome activity is found in the affected neurons [24]. This finding leads to the question of what causes accumulation of polyQ-Atxn7 and whether this accumulation is directly related to altered levels of ubiquitin in neuronal tissues in SCA7 patients.

SAGA functions in transcription and more

Atxn7 itself contains no enzymatic domains, but it bridges the HAT and DUB modules within the SAGA complex [8]. SAGA is highly conserved from yeast to human, both in the composition and organization of subunits [25-27]. By acetylating histones H3 and H4, and by removing ubiquitin from histone H2B, via enzymatic subunits Gcn5 and Usp22, respectively, SAGA facilitates transcriptional initiation [28, 29]. Therefore SAGA is best known for its functions as a transcriptional co-activator. However, both GCN5 and USP22 have non-histone substrates that impinge on transcription as well as other cellular processes. For example, Gcn5 and its sister protein, PCAF, acetylate the p53 tumor suppressor, affecting its functions in gene activation [30]. GCN5 and SAGA also regulate the activity of several transcription factors involved in metabolism (e.g. PGC1-α, PGC1-β) [30, 31]. USP22 DUB activity regulates ubiquitination and turnover of at least two components of the telomeric shelterin complex, thereby affecting genome integrity [32]. USP22 and its yeast ortholog, Ubp8, remove ubiquitin from transcriptional regulators as well, affecting their association with or activation of target genes [33, 34]. Although most studies focus on the transcriptional functions of SAGA in histone modification, the non-histone targets of Gcn5 and USP22 must also be considered in determining how polyQ-Atxn7 causes SCA7.

SAGA functions in neural development and SCA7

Genetic studies indicate that Gcn5 and SAGA may be particularly important for normal neural development. Deletion of *Gcn5* in mice leads to early embryonic lethality, but loss of Gcn5 HAT activity or decreased overall expression of Gcn5 leads to cranial neural tube closure defects and exencephaly [35, 36]. A loss-of-function mutation of the USP22 homologue in Drosophila, called *Nonstop*, leads to defects in neuronal projection [37].

Because Atxn7 bridges the HAT and DUB modules of SAGA, polyQ-Atxn7 could affect functions of either or both of these activities. Mice bearing polyQ-Atxn7 alleles provide a good model for human SCA7 disease [38]. In these SCA7 mouse models, neurodegeneration is accompanied by disruption of expression of neuronal genes [38] that are transcriptional targets of SAGA [39, 40]. Levels of Gcn5 HAT activity are decreased in polyQ-Atxn7 expressing cultured cells [41], suggesting that defects in Gcn5 functions caused by the Atxn7 mutant protein might underlie SCA7 disease development or progression. However, another study using a polyQ-Atxn7 transgenic mouse found that Gcn5 HAT activity was not adversely affected, and surprisingly, that higher levels of acetylated histones were associated with transcriptional down-regulation of Atxn7 and SAGA target genes [42]. These conflicting results suggest that polyQ-Atxn7 might cause either a loss-of-function or a gain-of-function in Gcn5 and SAGA.

To further address this question and to directly determine whether loss of Gcn5 functions exacerbates or improves the phenotype of SCA7 mouse models, Chen *et al.* [43] combined *Gcn5* mutations with polyQ expansions in Atxn7 that

cause moderate neurological phenotypes. Reduction of Gcn5 to 50% of normal levels accelerated both the onset and the progression of ataxia and neurodegeneration in the SCA7 mice. This finding clearly indicates that loss of SAGA functions is associated with SCA7 development. However, deletion of *Gcn5* specifically in Purkinje cells in the absence of polyQ-Atxn7 caused only very mild ataxia, suggesting that loss of Gcn5 HAT functions is not sufficient to induce SCA7 in a cell-autonomous fashion. Moreover, transcriptional down-regulation of known Atxn7 targets by polyQ-Atxn7 was not further affected by decreased levels of Gcn5 [43]. This work suggests that corruption of non-HAT, and likely non-transcriptional, functions of SAGA contribute to SCA7 development and progression. Since Gcn5 is required for full activity of USP22, depletion of Gcn5 might well hamper the functions of a DUB module already crippled by polyQ-Atxn7 (Figure 1). Decreased DUB activity could then contribute to the accumulation of ubiquitin observed in SCA7 cells.

Cell- or non-autonomous toxicity of polyQ-Atxn7?

Several recent findings suggest that the major toxicity of polyQ-Atxn7 comes not only from the affected neurons but also from surrounding cell types. Expressing polyQ-Atxn7 in either Purkinje neurons or Bergmann glia both cause cerebellar degeneration and ataxia [44, 45], suggesting polyQ-Atxn7 generates both cell- and non-autonomous toxicity towards the degenerating Purkinje cells in the cerebellum. Interestingly, major transcriptional alterations in the cerebellum were observed in surrounding interneurons and glia but not degenerating Purkinje neurons [46]. SAGA functions, both HAT and DUB related, could affect Bergmann glia-mediated non-autonomous toxicity in SCA7. Expression of polyQ-Atxn7 in human astrocytes was found to cause transcriptional down-regulation of a number of genes, including Reelin (*RELN*), which encodes for an extracellular matrix protein (McCullough *et al.* in press 2012). *RELN* has a well-documented role in the development and maintenance of Purkinje cells in the cerebellum. Furthermore, polyQ-Atxn7 expressing astrocytes display an increase in ubiquitinated H2B at the *RELN* gene promoter. This is consistent with a non-cell autonomous SAGA DUB-related defect that could contribute to the overall neuronal degeneration observed in SCA7 disease.

How polyQ-Atxn7 might lead to neurodegeneration in SCA7

PolyQ-Atxn7 likely has a dosage effect on toxicity. Earlier onset and more severe neurodegenerative phenotypes occur upon over expression of polyQ-Atxn7 and in the presence of long polyQ alleles, such as in the 266Q-SCA7 homozygote mouse [38, 47]. PolyQ-Atxn7 protein accumulates in degenerating tissues in SCA7 mice even though transcription of the polyQ-Atxn7 allele is not altered [38]. These observations suggest that polyQ-Atxn7 may have a longer half life and be cleared more slowly than wild type Atxn7. Since polyQ-Atxn7 but not wild type Atxn7 accumulates, a greater proportion of SAGA complexes would incorporate polyQ-Atxn7 over time, explaining both the dominance of the polyQ allele over the wild type allele and the increased toxicity as a function of age (Figure 1). Accumulation of ubiquitinated proteins due to reduced DUB function might result in less free ubiquitin for use in clearance of unfolded proteins, which in turn could aggravate neurotoxicity (Figure 2). The neuronal defects could be further exacerbated by the transcriptional functions of SAGA in the unfolded protein response and the ER-stress pathway [48, 49].

Increasing evidence shows that protein degradation has important roles in both neuronal development and in long-term synaptic plasticity [50]. The highly dynamic nature of synapses and the need for post-mitotic neurons to remain plastic and to constantly renew internal components to function properly makes them particularly dependent on an efficient ubiquitination system and protein turnover by the proteasome. For example, loss of Usp14, a proteasome-associated DUB enzyme, causes a reduction in ubiquitin pools that results in a neuromuscular developmental disorder in *ataxia* (ax^J) mice [51, 52]. Transgenic complementation of neuronal ubiquitin was reported to prevent the disease, indicating that ubiquitin homeostasis is an essential component of neuronal function [52]. Neurons are faced with the combined morphological

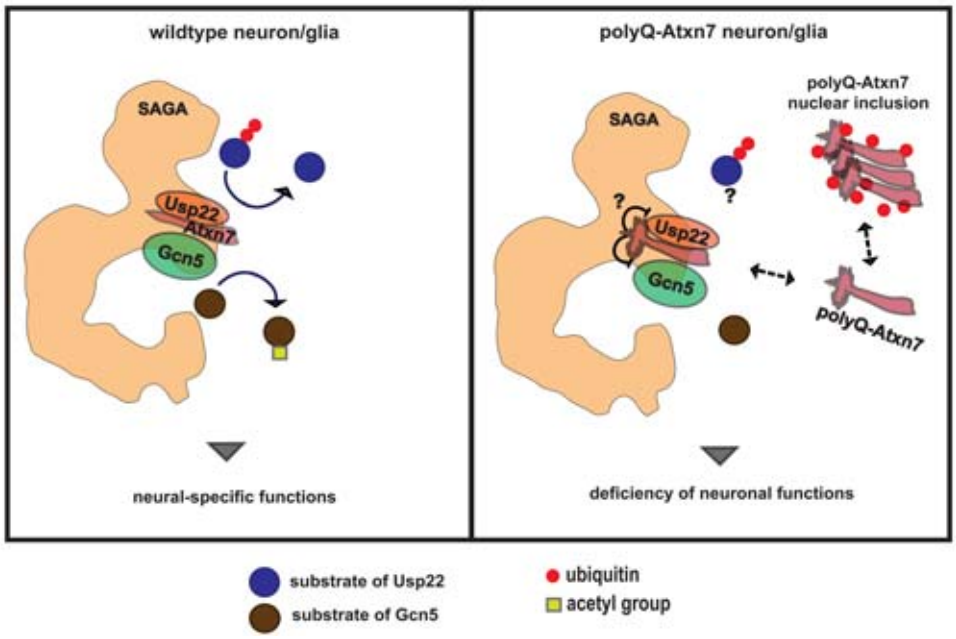

Figure 1. Model for progressive loss of SAGA functions in polyQ-Atxn7 mediated neuronal toxicity.
In wildtype cells, SAGA regulates neuronal functions via protein acetylation by Gcn5 and deubiquitination by Usp22. Since Atxn7 bridges these two catalytic activities, polyQ-Atxn7 incorporation into SAGA could affect both of these functions. The ratio of polyQ-Atxn7-containing SAGA would increase with age, as the polyQ protein, but not wild type Atxn7, accumulates. Neurons may be more sensitive to the loss of SAGA activity and/or the free ubiquitin pool and therefore more vulnerable to polyQ-Atxn7.

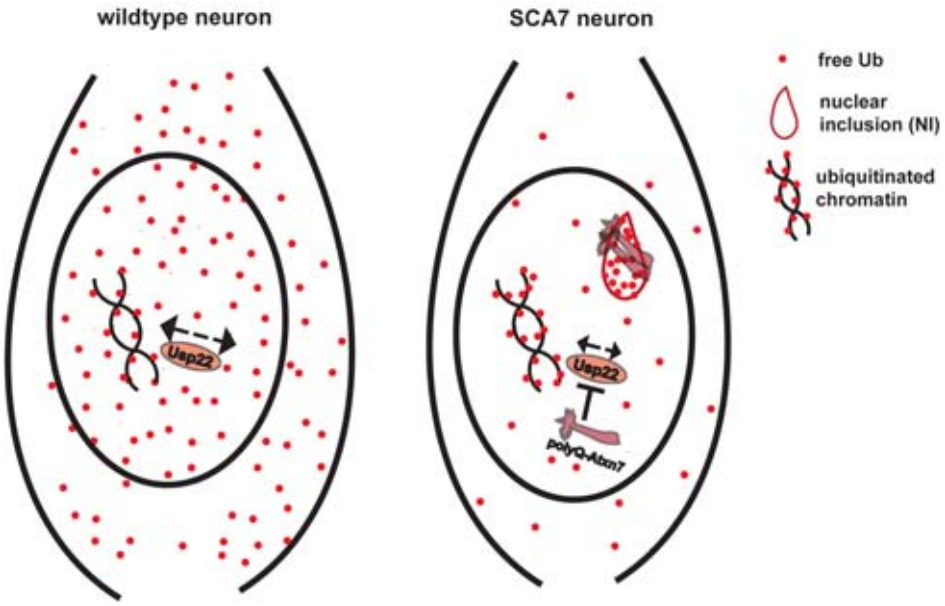

Figure 2. Model for how an unbalanced free ubiquitin pool contributes to SCA7 neurotoxicity. Homeostasis of a free ubiquitin pool may be associated with the dynamics of histone ubiquitination and deubiquitination. Since Usp22 is one of the DUBs that catalyze histone deubiquitination, Usp22 activity likely impacts the free ubiquitin pool. In SCA7 neurons, polyQ-Atxn7 might adversely affect Usp22 functions and disrupt the homeostasis of free ubiquitin. Since neurons maintain a higher free ubiquitin pool than other cell types, they may be especially sensitive to loss of Usp22 functions, leading to neural-specific toxicity and SCA7.

and biological challenge of having a comparatively large cell surface area, degrading surface proteins at a high rate, and then turning over locally or delivering these substrates to proteolytic machineries in compartmentalized spaces. The prevalence of neurodegenerative disorders, including polyQ diseases, that display changes in protein turnover seems to suggest that neurons are particularly susceptible to failures to clear aberrant proteins. The aggregation of free ubiquitin with polyQ-Atxn7 in nuclear inclusions would further worsen this problem (Figure 2). Although the ubiquitin pool might be affected in all cell types, neurons may be more vulnerable to this change due to their higher demand on the ubiquitin proteasome system [53, 54]. Since the overall proteasome activity in brain tissue decreases with aging [50], defective Usp22 DUB activity and putative alterations in ubiquitin pools in SCA7 neurons may contribute to the age-related onset of disease.

FUTURE PERSPECTIVE

Understanding the biochemical activities and the processes that are affected by the polyQ expansions in SCA7 and other triplet repeat diseases is crucially important to the development of new and better therapies. Our suggestion here that polyQ-Atxn7 might affect the DUB module in SAGA predicts that replacement DUBs, elevated ubiquitin levels or inhibiting ubiquitination pathways might slow the progression of neurodegeneration in SCA7. Our ideas can be tested in mice, by determining whether loss of Usp22 in the cerebellum leads to a SCA7 like phenotype. Going forward, it will also be important to determine whether the accumulation of specific ubiquitinated proteins is central to the cause of SCA7 or whether the level of free ubiquitin is more important to SCA7 neurotoxicity. Identifying protein targets of Usp22 and comparing the free ubiquitin pools in SCA7 mice, *Usp22* mutants, and double *Usp22*-SCA7 mutants will provide insights into these questions and determine whether ubiquitin-centric therapeutic interventions may be a potential way of treating not only SCA7 but also other related polyQ diseases.

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REFERENCES

1. David, G., Abbas, N., Stevanin, G., Durr, A., Yvert, G., Cancel, G., Weber, C., Imbert, G., Saudou, F., Antoniou, E., Drabkin, H., Gemmill, R., Giunti, P., Benomar, A., Wood, N., Ruberg, M., Agid, Y., Mandel, J.-L., and Brice, A. 1997, Nature Genetics,17, 65.
2. David, G., Durr, A., Stevanin, G., Cancel, G., Abbas, N., Benomar, A., Belal, S., Lebre, A. S., Abada-Bendib, M., Grid, D., Holmberg, M., Yahyaoui, M., Hentati, F., Chkili, T., Agid, Y., and Brice, A. 1998, Hum. Mol. Genet., 7, 165.
3. Giunti, P., Stevanin, G., Worth, P. F., David, G., Brice, A., and Wood, N. W. 1999, The American Journal of Human Genetics, 64, 1594.
4. Strom, A.-L., Jonasson, J., Hart, P., Brannstrom, T., Forsgren, L., and Holmberg, M. 2002, Gene, 285, 91.
5. Helmlinger, D., Hardy, S., Sasorith, S., Klein, F., Robert, F., Weber, C., Miguet, L., Potier, N., Van-Dorsselaer, A., Wurtz, J. M., Mandel, J. L., Tora, L., and Devys, D. 2004, Hum. Mol. Genet., 13, 1257.
6. Lam, Y. C., Bowman, A. B., Jafar-Nejad, P., Lim, J., Richman, R., Fryer, J. D., Hyun, E. D., Duvick, L. A., Orr, H. T., Botas, J., and Zoghbi, H. Y. 2006, Cell, 127, 1335.
7. Lim, J., Crespo-Barreto, J., Jafar-Nejad, P., Bowman, A. B., Richman, R., Hill, D. E., Orr, H. T., and Zoghbi, H. Y. 2008, Nature, 452, 713.
8. Zhao, Y., Lang, G., Ito, S., Bonnet, J., Metzger, E., Sawatsubashi, S., Suzuki, E., Le Guezennec, X., Stunnenberg, H. G., Krasnov, A., Georgieva, S. G., Schüle, R., Takeyama, K.-I., Kato, S., Tora, L., and Devys, D. 2008, Molecular Cell, 29, 92.
9. Ansorge, O., Giunti, P., Michalik, A., Van Broeckhoven, C., Harding, B., Wood, N., and Scaravilli, F. 2004, Ann. Neurol., 56, 448.
10. Cummings, C. J., Dahle, E. J., and Zoghbi, H. Y. 1998, Am. J. Med. Genet., 78, 176.
11. Davies, S. W., Beardsall, K., Turmaine, M., DiFiglia, M., Aronin, N., and Bates, G. P. 1998, Lancet, 351, 131.
12. Koyano, S., Uchihara, T., Fujigasaki, H., Nakamura, A., Yagishita, S., and Iwabuchi, K. 1999, Neurosci. Lett., 273, 117.

13. Lunkes, A. and Mandel, J. L. 1997, Nat. Med., 3, 1201.
14. Mauger, C., Del-Favero, J., Ceuterick, C., Lubke, U., van Broeckhoven, C., and Martin, J. 1999, Brain Res. Mol. Brain Res., 74, 35.
15. Ross, C. A. 1997, Neuron, 19, 1147.
16. Giuditta, A., Kaplan, B. B., van Minnen, J., Alvarez, J., and Koenig, E. 2002, Trends Neurosci., 25, 400.
17. Mabb, A. M. and Ehlers, M. D. 2010, Annu. Rev. Cell Dev. Biol., 26, 179.
18. Kaiser, S. E., Riley, B. E., Shaler, T. A., Trevino, R. S., Becker, C. H., Schulman, H., and Kopito, R. R. 2011, Nat. Methods, 8, 691.
19. Imai, Y., Soda, M., and Takahashi, R. 2000, J. Biol. Chem., 275, 35661.
20. Kishino, T., Lalande, M., and Wagstaff, J. 1997, Nat. Genet., 15, 70.
21. Saigoh, K., Wang, Y. L., Suh, J. G., Yamanishi, T., Sakai, Y., Kiyosawa, H., Harada, T., Ichihara, N., Wakana, S., Kikuchi, T., and Wada, K. 1999, Nat. Genet., 23, 47.
22. Wilson, S. M., Bhattacharyya, B., Rachel, R. A., Coppola, V., Tessarollo, L., Householder, D. B., Fletcher, C. F., Miller, R. J., Copeland, N. G., and Jenkins, N. A. 2002, Nat. Genet., 32, 420.
23. Wintermeyer, P., Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Berg, D., Becker, G., Leroy, E., Polymeropoulos, M., Berger, K., Przuntek, H., Schols, L., Epplen, J. T., and Riess, O. 2000, Neuroreport, 11, 2079.
24. Bowman, A. B., Yoo, S. Y., Dantuma, N. P., and Zoghbi, H. Y. 2005, Hum. Mol. Genet., 14, 679.
25. Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. 1997, Genes Dev., 11, 1640.
26. Kusch, T., Guelman, S., Abmayr, S. M., and Workman, J. L. 2003, Mol. Cell Biol., 23, 3305.
27. Martinez, E., Kundu, T. K., Fu, J., and Roeder, R. G. 1998, Journal of Biological Chemistry, 273, 23781.
28. Baker, S. P. and Grant, P. A. 2007, Oncogene, 26, 5329.
29. Rodriguez-Navarro, S. 2009, EMBO Rep., 10, 843.
30. Kelly, T. J., Lerin, C., Haas, W., Gygi, S. P., and Puigserver, P. 2009, Journal of Biological Chemistry, 284, 19945.
31. Lerin, C., Rodgers, J. T., Kalume, D. E., Kim, S. H., Pandey, A., and Puigserver, P. 2006, Cell Metabolism, 3, 429.
32. Atanassov, B. S., Evrard, Y. A., Multani, A. S., Zhang, Z., Tora, L., Devys, D., Chang, S., and Dent, S. Y. 2009, Molecular Cell, 35, 352.
33. Atanassov, B. S. and Dent, S. Y. 2011, EMBO Rep., 12, 924.
34. Wilson, M. A., Koutelou, E., Hirsch, C., Akdemir, K., Schibler, A., Barton, M. C., and Dent, S. Y. 2011, Mol. Cell Biol., 31, 3126.
35. Bu, P., Evrard, Y. A., Lozano, G., and Dent, S. Y. 2007, Molecular & Cellular Biology, 27, 3405.
36. Lin, W., Zhang, Z., Srajer, G., Chen, Y. C., Huang, M., Phan, H. M., and Dent, S. Y. 2008, Developmental Dynamics, 237, 928.
37. Weake, V. M., Lee, K. K., Guelman, S., Lin, C. H., Seidel, C., Abmayr, S. M., and Workman, J. L. 2008, EMBO J., 27, 394.
38. Yoo, S.-Y., Pennesi, M. E., Weeber, E. J., Xu, B., Atkinson, R., Chen, S., Armstrong, D. L., Wu, S. M., Sweatt, J. D., and Zoghbi, H. Y. 2003, Neuron, 37, 383.
39. Palhan, V. B., Chen, S., Peng, G.-H., Tjernberg, A., Gamper, A. M., Fan, Y., Chait, B. T., La Spada, A. R., and Roeder, R. G. 2005, Proceedings of the National Academy of Sciences of the United States of America,102, 8472.
40. Peng, G.-H. and Chen, S. 2007, Human Molecular Genetics,16, 2433.
41. Hughes, R. E., Lo, R. S., Davis, C., Strand, A. D., Neal, C. L., Olson, J. M., and Fields, S. 2001, Proc. Natl. Acad. Sci. USA, 98, 13201.
42. Helmlinger, D., Hardy, S., Abou-Sleymane, G., Eberlin, A., Bowman, A. B., Gansmüller, A., Picaud, S., Zoghbi, H. Y., Trottier, Y., Tora, L., and Devys, D. 2006, PLoS Biol, 4:e67
43. Chen, Y. C., Gatchel, J. R., Lewis, R. W., Mao, C. A., Grant, P. A., Zoghbi, H. Y., and Dent, S. Y. 2011, Hum. Mol. Genet., 21, 394.
44. Custer, S. K., Garden, G. A., Gill, N., Rueb, U., Libby, R. T., Schultz, C., Guyenet, S. J., Deller, T., Westrum, L. E., Sopher, B. L., and La Spada, A. R. 2006, Nat. Neurosci., 9, 1302.

45. Garden, G. A., Libby, R. T., Fu, Y.-H., Kinoshita, Y., Huang, J., Possin, D. E., Smith, A. C., Martinez, R. A., Fine, G. C., Grote, S. K., Ware, C. B., Einum, D. D., Morrison, R. S., Ptacek, L. J., Sopher, B. L., and Spada, A. R. L. 2002, The Journal of Neuroscience, 22, 4897.
46. Gatchel, J. R., Watase, K., Thaller, C., Carson, J. P., Jafar-Nejad, P., Shaw, C., Zu, T., Orr, H. T., and Zoghbi, H. Y. 2008, Proceedings of the National Academy of Sciences, 105, 1291.
47. La Spada, A. R., Fu, Y.-H., Sopher, B. L., Libby, R. T., Wang, X., Li, L. Y., Einum, D. D., Huang, J., Possin, D. E., Smith, A. C., Martinez, R. A., Koszdin, K. L., Treuting, P. M., Ware, C. B., Hurley, J. B., Ptácek, L. J., and Chen, S. 2001, Neuron, 31, 913.
48. Nagy, Z., Riss, A., Romier, C., le Guezennec, X., Dongre, A. R., Orpinell, M., Han, J., Stunnenberg, H., and Tora, L. 2009, Mol. Cell Biol., 29, 1649.
49. Schroder, M. and Kaufman, R. J. 2005, Annu. Rev. Biochem., 74, 739.
50. Tai, C. Y., Mysore, S. P., Chiu, C., and Schuman, E. M. 2007, Neuron, 54, 771.
51. Anderson, C., Crimmins, S., Wilson, J. A., Korbel, G. A., Ploegh, H. L., and Wilson, S. M. 2005, J. Neurochem., 95 ,724,
52. Chen, P. C., Qin, L. N., Li, X. M., Walters, B. J., Wilson, J. A., Mei, L., and Wilson, S. M. 2009, J. Neurosci., 29, 10909.
53. Fierz, B., Chatterjee, C., McGinty, R. K., Bar-Dagan, M., Raleigh, D. P., and Muir, T. W. 2011, Nat. Chem. Biol., 7, 113.
54. Staropoli, J. F. and Abeliovich, A. 2005, J. Mol. Neurosci., 27, 175.