A multifunctional, multi-pathway intracellular localization signal in Huntingtin

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The adult-onset neurodegenerative disorder Huntington's Disease (HD) is caused by a polyglutamine repeat expansion mutation near the N-terminus of the 350 kDa protein, huntingtin. Despite the widespread expression of huntingtin in all cells, neurons within the striatum and cerebral cortex are selectively vulnerable to the toxicity of mutant huntingtin. The earliest transgenic mouse model of HD, termed R6/2, expresses only a short amino-terminal fragment of huntingtin (1–81 amino acids). This region contains the polyglutamine tract, and produces an extremely severe pathological phenotype, far greater than is observed in full-length huntingtin mouse models. In patients, polyglutamine tract length is strongly correlated with disease onset, with a greater number of repeats being associated with earlier development of symptoms. This is also true for R6/2 mice – up to a particular threshold. At repeat lengths beyond 200, the life expectancy of the R6/2 mice shows a paradoxical improvement, reversing the previous trend. This phenomenon may be related to the way the small, 1–81 amino acid (< 10KDa) fragment enters the nucleus. The diffusion limit of the nuclear pore complex (NPC) is roughly defined, but has been noted as approximately 50–60 kDa, with larger proteins requiring a nuclear localization signal to enter by a mechanism of facilitated diffusion. The 1–81 fragment falls far below this threshold, granting it unrestricted access to the nuclear compartment. If nuclear translocation of mutant huntingtin is detrimental, this free and unregulated diffusion could be responsible for the severity of the R6/2 model. Thus, a slowed nuclear import therapeutically and the potential impacts of targeting huntingtin localization signal near the n-terminus of huntingtin. We have recently reported the characterization of the first definitive nuclear localization signal (NLS) within huntingtin. This non-classical, proline-tyrosine, or PY-NLS, found between amino acids 174–207, possesses a unique structured region that is required for recognition of the sequence by the import receptors karyopherin β1 and β2 (also known as importin beta1 and transportin). We have named this structured region the “intervening sequence” (IVS) due to its location between the consensus epitopes of the NLS that are comprised of a basic region, a downstream single arginine and the proline-tyrosine. This type of NLS is not unique to huntingtin, and is seen in the mRNA export factor, NXF1. The NXF1 PY-NLS can function through four karyopherin family pathways, including karyopherin β1 and β2, and does have a long stretch of residues between the NLS epitopes. In isolation, the IVS is capable of localizing to the cytoplasm, suggesting that it may regulate the activity of the NLS by targeting huntingtin to an insoluble phase. Our hope is that this data will guide the development of genetic tools for future exploration and proof of principle of the pathogenic link between huntingtin nuclear import and toxicity.

Nuclear accumulation of the polyglutamine-expanded mutant huntingtin protein remains one of the most predictive cell biological phenotypes of Huntington’s disease (HD) progression in patient brain samples and mouse models of the disease. Yet, the relationship between huntingtin nuclear import, neuronal dysfunction and toxicity is not fully understood and it remains unclear whether nuclear accumulation is required for disease onset. Here, we discuss several studies that have guided current understanding of this subject, and highlight our recent data detailing the discovery of a karyopherin β1/β2-type nuclear localization signal near the N-terminus of huntingtin. This signal can function through multiple pathways of nuclear import, and may also be responsible for huntingtin import into the primary cilium. This work represents a significant step forward in our knowledge of the regulatory pathways that govern huntingtin nuclear accumulation and will allow direct examination of both normal and mutant huntingtin nuclear function. This work also suggests a re-examination of the cell biology of any protein that contains a multi-pathway nuclear localization signal. The possibility of targeting huntingtin nuclear import therapeutically and the potential impacts of such a strategy for the treatment of HD are also discussed.

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or inhibited diffusion, as would be expected with the addition of an extremely long polyglutamine tract, could manifest as reduced pathogenicity in a small fragment context. Consistent with this hypothesis, immunohistochemistry of brain samples from mice bearing longer repeats show a reduction in the formation of nuclear aggregates, and an increase in those that form in the cytoplasm, indicating a potential loss of ability of the huntingtin fragments to traverse the nuclear pore. In larger fragment or full-length models, huntingtin is capable of nuclear entry via the PY-NLS sequence and not by simple diffusion alone. With this added regulation there is greater potential to control the timing of nuclear entry, thus delaying the onset of cellular pathology. Severe toxicity of HD mouse models is limited to the 1–81 R6/2 or N17122 (1–171 fragment) models. Intriguingly, the huntingtin NLS starts at residue 174.

Consistent with its ability to shuttle from one compartment to the other, huntingtin is also equipped with two CRM1-dependent nuclear export signals: one toward the carboxyl-terminus of the protein, and one at the extreme amino-terminus within the N17 regulatory domain (Maiuri et al., in press). In addition to binding to CRM1, N17 also acts as an endoplasmic reticulum (ER) targeting signal that prevents huntingtin from accumulating in the nucleus under normal cellular conditions. During cell stress, N17 becomes phosphorylated at serines 13 and 16 causing release of huntingtin from the ER and enhanced nuclear entry. Thus, it is unclear if the nuclear accumulation of huntingtin observed in degenerating neurons is simply a consequence of persistent cell stress, or a toxic process in itself. A bacterial artificial chromosome full-length huntingtin transgenic model (BACHD) has been developed that is modified at serines 13 and 16 to mimic constitutive phosphorylation (S-D mutation) or constitutive phospho-resistance (S-A mutation). Although phosphorylation (or phospho-mimicry) of huntingtin at N17 is predicted to enhance the protein’s nuclear translocation, in this model, the S-D mutation was protective. Consistent with these genetic experiments, treatments that increase the phosphorylation of N17 in full-length mouse models, such as the ganglioside GM1, show striking phenotypic reversal. Thus, the relationship between nuclear import and mutant huntingtin toxicity remains unclear. Rather than nuclear translocation, the phosphoN17-status or sub-nuclear localization of huntingtin to discreet chromatin-dependent puncta may confer toxicity. Alternatively, it may be overly simplistic to view either the nucleus or the cytoplasm as the sole site of toxicity. Instead, the primary issue may be an inability to shuttle efficiently between the two compartments, causing a breakdown in cellular signaling. The stress response switch mediated by huntingtin may therefore be stuck in an on or off state, losing its ability to dynamically switch between the two. The appealing aspect of this hypothesis is that it implies that this huntingtin function is more important as the human brain ages, during which time metabolic stresses are known to increase, along with decreasing mitochondrial efficiency.

Elucidation of the mechanisms of huntingtin nuclear and cytoplasmic shuttling will shed further light on whether limiting nuclear accumulation has therapeutic potential. Many nuclear import pathways are not valid drug targets, due to lack of specificity in NLS-import factor interactions; however, the huntingtin NLS has a unique structured IVS that could be exploited as a binding region for small molecules. Still, this avenue should be pursued with caution. It was recently reported that huntingtin has a role in ciliogenesis, and our lab has discovered that huntingtin is capable of entering the body of the primary cilium (Maiuri et al., in press).

Cilial entry and nuclear entry have a surprising amount in common. Proteins that form the nuclear pore complex create a permeability or diffusion barrier at the base of the primary cilium, and the import receptor karyopherin β2 has been found to mediate cilial entry through recognition of PY-NLS sequences. Like the nuclear pore complex, some nucleoporins, or nups, can be found at the cilial permeability barrier even though the cilial barrier is not membrane-based. Thus, multi-pathway localization signals may exist in proteins that use the nucleoporin and Ran GTP/GDP gradient to traffic in and out of the cilium as well as the nucleus. Therefore, although disrupting mutant huntingtin nuclear entry may combat the toxic effects that cause HD, it could also inhibit the cilial function of the protein.

The primary cilium is a non-motile singular organelle in neurons, used as an extra-synaptic antenna for soluble signaling through G-protein coupled receptors (GPCRs). While vesicles are not found within the cilium, vesicular motor proteins and complexes exist within the cilium to move internalized GPCRs into the cell. The function of huntingtin in the cilium may be similar to its role in vesicular trafficking. Huntingtin acts as a scaffolding protein that connects vesicles to kinesin, as well as to the p150 glued subunit of dynactin, an activator of dynein. These microtubule motor proteins are also required for intraflagellar transport along the axoneme of the cilium, which is composed of tubulin bundles.

Our work to date indicates that the steady-state localization of huntingtin is either at trafficking early endosomes, or the endoplasmic reticulum. Huntingtin only releases from the ER during cell stress that involves low ATP, where the soluble protein then translocates to the nucleus and cilium. When mutant huntingtin is present, even heterozygously, cells are unable to recover from stresses that involve an ATP-capping response at the actin cytoskeleton within the nucleus. ATP is also utilized by motor proteins in the primary cilium. We hypothesize that the transient localization of huntingtin to the nucleus and cilium may be required for an optimal cell stress response in neurons.

It is still not clearly understood why some proteins have multiple nuclear transport signals, and why some signals can use multiple pathways of nuclear, and now cilial entry. These karyopherin/importin proteins are now known to have other important cell biological functions aside from just carrying cargo to or from the nucleus. As the knowledge of the biology of these import factors increases, so then will the role of these multifunctional, multipathway transport signals in proteins.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
References
1. The Huntington’s Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. Cell 1993; 72:971-83; PMID:8458085; http://dx.doi.org/10.1016/0092-8674(93)90585-E.
2. Andrade MA, Bork P. HEAT repeats in the Huntington’s disease protein. Nat Genet 1995; 11:115-6; PMID:755032; http://dx.doi.org/10.1038/ng1095-115.
3. Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson JP Jr. Neuropathological classification of Huntington’s disease. J Neuropathol Exp Neurol 1985; 44:579-77; PMID:2292359; http://dx.doi.org/10.1097/00005072-198511000-00003.
4. Caviston JP, Holzbaur EL. Huntington as an essential integrator of intracellular vesicular trafficking. Trends Cell Biol 2009; 19:147-55; PMID:19269181; http://dx.doi.org/10.1016/j.tcb.2009.01.005.
5. Truant R, Arwal RS, Butturk N. Nucleocytoplasmic trafficking and transcription effects of huntingtin in Huntington’s disease. Prog Neurobiol 2007; 83:211-27; PMID:17240517; http://dx.doi.org/10.1016/j.pneurobio.2006.11.004.
6. Smith R, Brundin P, Li JY. Synaptic dysfunction in Huntington’s disease: a new perspective. Cell Mol Life Sci 2005; 62:1901-12; PMID:15968465; http://dx.doi.org/10.1007/s00018-005-5084-5.
7. Kwan W, Träger U, Davalos D, Chou A, Bouchard J, Andre R, et al. Mutant huntingtin impairs immune cell migration in Huntington disease. J Clin Invest 2012; 122:4377-47; PMID:23160193; http://dx.doi.org/10.1172/JCI64484.
8. Munsie L, Caron N, Arwal RS, Marsden I, Wild EJ, Bamburg JR, et al. Mutant huntingtin causes defective actin remodeling during stress: defining a new role for transglutaminase 2 in neurodegenerative disease. Hum Mol Genet 2011; 20:1937-51; PMID:21355047; http://dx.doi.org/10.1093/hmg/ddt075.
9. Arwal RS, Truant R. A stress sensitive ER membrane-association domain in Huntington protein defines a potential role for Huntington in the regulation of autophagy. Autophagy 2008; 4:91-3; PMID:17986868.
10. De Rooij KE, Dorsman JC, Smoor MA, Den Ronaldo intranuclear inclusions and dystrophic neurites. J Neuropathol Exp Neurol 1985; 44:579-77; PMID:2292359; http://dx.doi.org/10.1097/00005072-198511000-00003.
11. Kwan W, Träger U, Davalos D, Chou A, Bouchard J, Andre R, et al. Mutant huntingtin impairs immune cell migration in Huntington disease. J Clin Invest 2012; 122:4377-47; PMID:23160193; http://dx.doi.org/10.1172/JCI64484.
12. Munsie L, Caron N, Arwal RS, Marsden I, Wild EJ, Bamburg JR, et al. Mutant huntingtin causes defective actin remodeling during stress: defining a new role for transglutaminase 2 in neurodegenerative disease. Hum Mol Genet 2011; 20:1937-51; PMID:21355047; http://dx.doi.org/10.1093/hmg/ddt075.
13. Arwal RS, Truant R. A stress sensitive ER membrane-association domain in Huntington protein defines a potential role for Huntington in the regulation of autophagy. Autophagy 2008; 4:91-3; PMID:17986868.
14. DiFiglia M, Sapp E, Chase KO, Davies SW, Bax G, Vonsattel JP, et al. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 1997; 277:1990-3; PMID:9302293; http://dx.doi.org/10.1126/science.277.5354.1990.
15. Van Raamsdonk JM, Murphy Z, Slow EJ, Leavitt BR, Hayden MR. Selective degeneration and nuclear localization of mutant huntingtin in the YAC128 mouse model of Huntington disease. Hum Mol Genet 2005; 14:3823-35; PMID:16278256; http://dx.doi.org/10.1093/hmg/ddi277.
16. Desmond CR, Arwal RS, Xia J, Truant R. Identification of a Karyopherin β1/B2 Proline-Tyrrosine Nuclear Localization Signal in Huntington Protein. J Biol Chem 2012; 287:39626-33; PMID:23012356; http://dx.doi.org/10.1074/jbc.M112.142179.
17. Xu D, Farmer A, Chook YM. Recognition of nuclear targeting signals by Karyopherin-β proteins. Curr Opin Struct Biol 2010; 20:782-90; PMID:20951026; http://dx.doi.org/10.1016/j.sbi.2010.09.008.
18. Zhang XC, Sannerby N, Fontoura RM, Chook YM. Evolutionary development of redundant nuclear localization signals in the mRNA export factor NXF1. Mol Biol Cell 2011; 22:4657-68; PMID:21965294; http://dx.doi.org/10.1099/mbe.0.001322.
19. Manguini L, Sarathivam K, Sell M, Cozens B, Harper A, Hetherington C, et al. Eum 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 1996; 87:493-506; PMID:8892020; http://dx.doi.org/10.1016/0092-8674(96)82015-4.
20. Andrew SE, Goldberg YP, Kremer B, Telenius H, Theilmann J, Adam S, et al. The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington’s disease. Nat Genet 1993; 4:398-403; PMID:8401859; http://dx.doi.org/10.1093/ng/8.4.398.
21. Morton AJ, Glynn D, Leavens W, Zheng Z, FauI RL, Skepper JN, et al. Paradoxical delay in the onset of disease caused by super-long CAG repeat expansions in R6/2 mice. Neurobiol Dis 2009; 33:331-41; PMID:19308848; http://dx.doi.org/10.1016/j.nbd.2008.08.015.
22. Dragantis I, Goldwitz D, Del Mar N, Deng YP, Meade CA, Liu L, et al. CAG repeat length > or = 135 attenuate the phenotype in the R6/2 Huntington’s disease transgenic mouse. Neurobiol Dis 2009; 33:315-30; PMID:19027857; http://dx.doi.org/10.1016/j.nbd.2008.10.009.
23. Terry LJ, Shows EB, Wente SR. Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. Science 2007; 318:1412-6; PMID:17684861; http://dx.doi.org/10.1126/science.1142204.
24. Meade CA, Liu J, et al. CAG repeat length > or = 135 attenuate the phenotype in the R6/2 Huntington’s disease transgenic mouse. Neurobiol Dis 2009; 33:315-30; PMID:19027857; http://dx.doi.org/10.1016/j.nbd.2008.10.009.
25. Morton AJ, Glynn D, Leavens W, Zheng Z, FauI RL, Skepper JN, et al. Paradoxical delay in the onset of disease caused by super-long CAG repeat expansions in R6/2 mice. Neurobiol Dis 2009; 33:331-41; PMID:19308848; http://dx.doi.org/10.1016/j.nbd.2008.08.015.
26. Scott RJ, Cairo LV, Van de Vosse DW, Wozniak RW. The role of karyopherins in the regulation of nuclear import and export. Nat Rev Mol Cell Biol 2011; 12; PMID:21623356; http://dx.doi.org/10.1038/nchembio.582.
27. Xu D, Farmer A, Chook YM. Recognition of nuclear targeting signals by Karyopherin-β proteins. Curr Opin Struct Biol 2010; 20:782-90; PMID:20951026; http://dx.doi.org/10.1016/j.sbi.2010.09.008.
28. Scott RJ, Cairo LV, Van de Vosse DW, Wozniak RW. The nuclear export factor Xpo1p targets Madlp to kinetochores in yeast. J Cell Biol 2009; 184:21-9; PMID:19139260; http://dx.doi.org/10.1083/ jcb.200804098.
29. Scott RJ, Cairo LV, Van de Vosse DW, Wozniak RW. The nuclear export factor Xpo1p targets Madlp to kinetochores in yeast. J Cell Biol 2009; 184:21-9; PMID:19139260; http://dx.doi.org/10.1083/jcb.200804098.