required by the fact they are regulated by small molecule binding. The somewhat larger displacement proposed for TorS (Moore and Hendrickson, 2009) is not unreasonable since it binds a regulatory protein, TorT, and the resulting protein-protein interaction could perhaps generate enough binding free energy to drive larger changes in side chain and ridges-grooves interactions. Second, transmembrane signals in bacterial receptors must span distances of 150 Å or more from the periplasmic ligand binding site to the cytoplasmic domain, and thus must be transmitted over a remarkably long distance. To a first approximation, the H-bonding framework of an α helix is incompressible along the helix axis, ensuring that a piston force pushing on one end of a helix will be faithfully transmitted throughout the entire helix length. By contrast, helix bends, rotations, or tilts can be more easily damped by long-range helix flexibility over these distances. Third, a small 1-2 Å displacement is large enough to directly regulate the on-off switching of a kinase active site, or trigger a larger structural rearrangement in a signal conversion module such as the HAMP domain. Thus, it appears likely that chemoreceptors and His kinase receptors have retained the same piston mechanism of transmembrane signaling for good biophysical reasons.

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Polyglutamine Dances the Conformational Cha-Cha-Cha

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While polyglutamine repeats appear in dozens of human proteins, high-resolution structural analysis of these repeats in their native context has eluded researchers. Kim et al. now describe multiple crystal structures and demonstrate that polyglutamine in huntingtin dances through multiple conformations.

There are 66 human proteins with a homopolymeric stretch of five glutamines or more. The overrepresentation of polyglutamine (polyQ)-containing proteins in transcription-related processes suggests a critical function for these repeats (Butland et al., 2007). At least 9 of these 66 proteins have a polyQ stretch that, when expanded beyond a critical threshold, misfold, aggregate, and cause neurodegenerative diseases. Although the structural basis that underlies the toxicity of proteins with expanded polyQ repeats is not clear, numerous laboratories have hypothesized that a variety of misfolded conformers, including monomers, oligomers, and fibrils, are the toxic culprits.

Into this debate enters the heroic crystallography feat of Kim et al. (2009). The authors solved seven independent crystal structures of a Q_{17}-containing exon1 fragment of wild-type huntingtin (Htt^{ex1}), a multifunctional protein that, when mutated in the polyQ stretch ($>Q_{36}$), causes a devastating neurodegenerative disorder called Huntington's chorea (chorea, derived from Greek, describes the involuntary dance-like movements of Huntington's patients). Reminiscent of the dance-like contortions of affected patients, the

wild-type polyQ stretch in Htt^{ex1} was surprisingly crystallized in multiple conformational contortions, most convincingly forming α helices that varied from 1–15 polyQ residues in length (Figure 1A). Although the structure of the polyQ sequences C-terminal to these helices was not always well resolved in the crystal structures, the authors suggest that these sequences likely adopted a random coil or extended-loop conformation. The sequences surrounding the polyQ stretch, the structures of which have also been contested, generally demonstrated less conformational flexibility. The 17 amino acids N-terminal to the polyQ sequence in Htt^{ex1} (N^{17}) were invariably α-helical in every structure that was solved, consistent with structure prediction programs and circular dichroism (CD) spectroscopy (Atwal et al., 2007). C-terminal to the polyQ region is a polyproline stretch, which formed a classic proline helix, also as suggested by CD experiments (Darnell et al., 2007). Interestingly, the polyproline stretch was either straight or kinked (Figure 1B), suggesting that this sequence in huntingtin may itself exhibit some conformational flexibility.

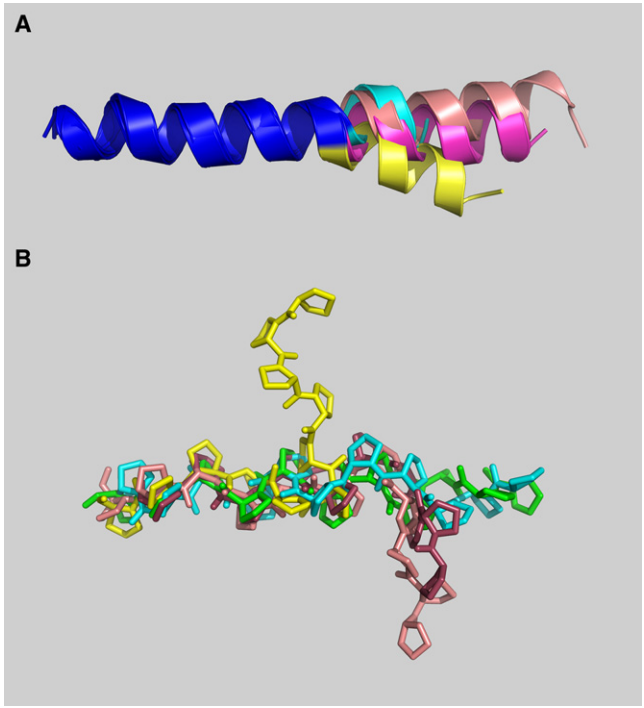

Figure 1. Conformational Cha-cha-cha: X-Ray Crystallography Reveals That PolyQ and Polyproline Adopt Multiple Conformations in Htt Exon1

(A) Four α helices are shown. Each extends from the N-terminal residue of the N^{17} region (Met 371-Phe 387) of Htt Exon1 (blue) and continues as α helix for a varying number of glutamine residues (cyan = 5, yellow = 9, magenta = 12, and salmon = 15). Glutamines C-terminal to the α-helical structured residues may adopt other conformations, including random coil, extended loop, or β strand.

(B) Five of the seven observed polyproline regions of Htt Exon1 are shown superimposed on their five C-terminal residues. Note that all demonstrate a proline-helix conformation, but some are kinked while others are extended. This figure was generated using PyMol (www.pymol.org).

Before interpreting and digesting this wealth of structural information, it is worth reflecting upon this astounding technical feat. Since the huntingtin gene was cloned more than sixteen years ago, numerous laboratories have attempted and failed to determine the structure of various huntingtin fragments. Indeed, this is the first crystal structure of any polyQ-containing (>Q$_{10}$) protein in its native protein context. The fact that the polyQ stretch in the Htt^{ex1} fragment adopts different conformations within the asymmetric unit of each crystal that the authors solved, combined with the fact that Kim et al. (2009) analyzed diffraction from 30 crystals and obtained structures for seven crystal forms, speaks to the daunting nature of the entire effort. The authors demonstrated significant insight by recognizing that the structures of the N^{17} and polyproline regions are relatively constant, while the polyQ region varied.

The conformational flexibility of the polyQ region in Htt^{ex1} raises several interesting questions about the functional role of these stretches. For example, of the 66 human proteins with ≥ Q$_5$ stretch, approximately half (including all proteins associated with polyQ-expansion disease) demonstrate significant length polymorphisms in the polyQ stretch in the normal human population. Are polyQ stretches only conformationally flexible in the proteins with length polymorphism? A protein that must be functional within a wide range of polyQ lengths may have to consequently demonstrate significant conformational flexibility in this region. How does this conformational flexibility assist in cellular functions? For example, does the overrepresentation of polyQ proteins in transcription-related processes suggest conformational flexibility is especially important for these processes? Another interesting question raised by this study is whether the polyQ stretch jumps between defined conformations (Nagai et al., 2007; Tuinstra et al., 2008) or fluidly flows through conformational space. Because Kim et al. (2009) observed a wide range of conformations for the polyQ stretch, one may assume that fluid conformational sampling may predominate. On the other hand, it is hard to imagine how Htt^{ex1} crystallized if there was not at least a limited set of conformations that the polyQ stretch samples.

From the perspective of neurodegenerative diseases, it is interesting to speculate whether the conformational sampling of space by the polyQ region increases, decreases, or stays the same when the polyQ stretch expands into the mutant (>Q$_{36}$) range. For example, while the structure of fully aggregated fibrillar polyQ in many proteins is composed predominantly of β sheet, Kim et al. (2009) did not observe this conformation in the crystal structures of wild-type Htt^{ex1}. Does this conformation exist among the portions of polyQ in Htt^{ex1}, whose electron density was unresolved by Kim et al. (2009)? Alternatively, does this β strand/sheet conformation emerge only in monomers of mutant Htt^{ex1} (>Q$_{36}$) or only upon aggregation? Notably, there is evidence that polyQ in monomeric mutant Htt^{ex1} can adopt a collapsed β sheet conformation (Nagai et al., 2007). Further, while a wide range of aggregate morphologies for mutant Htt^{ex1} species exists (Wacker et al., 2004), it is unknown whether a single conformation of polyQ in monomeric mutant Htt^{ex1} leads to a single type of aggregated species or, alternatively, whether a single monomeric conformation can produce all observed aggregate species. While a recent study with monoclonal antibodies strongly implicated the existence of multiple monomeric polyQ

conformations in mutant Htt^{ex1} (Legleiter et al., 2009), Kim et al. (2009) provide direct structural evidence of this, suggesting that, at least in principle, each conformation may seed a unique type of aggregate.

Even if we fully understood how different monomeric conformations of polyQ in Htt^{ex1} lead to various aggregated species, the questions of which species contribute to neurotoxicity and how they do it are still open questions. Kim et al. (2009) propose two general mechanisms for polyQ-mediated toxicity. By one mechanism, the expanded polyQ stretch adopts a de novo conformation that mediates toxicity or is the precursor to a toxic species. By the second mechanism, the expanded polyQ stretch is largely unstructured but presents a very large linear binding surface for proteins with a polyQ affinity. The structures from Kim et al. (2009) leave open the possibility that either mechanism may be correct.

The study by Kim et al. (2009) also provides interesting insight into the relationship between the polyQ stretch and the surrounding sequences in Htt^{ex1}. The N^{17} sequence, which is important for the subcellular localization of Htt^{ex1} and is highly conserved (100% similarity) in all vertebrate species (Atwal et al., 2007), was invariably α-helical in all solved structures. Interestingly, the N^{17} α-helix appears to "bleed" into the C-terminal adjacent polyQ region, causing 1–15 glutamines to participate in the extended α helix (Figure 1A). The structural data from Kim et al. (2009) also hint that the polyQ repeat in Htt^{ex1} may be influenced by the C-terminal polyproline region. Because Htt^{ex1} may be more aggregation prone (and possibly more toxic) when the polyQ region is more compact, it is interesting to speculate whether the polyproline region may serve both its known function as a protein-interaction domain and a less-appreciated function as a protector against polyQ conformational collapse. Indeed, this structural explanation may account for why Htt^{ex1} with the polyproline stretch is less toxic and aggregation prone than Htt^{ex1} without this sequence (Bhattacharyya et al., 2006; Darnell et al., 2007; Duennwald et al., 2006). Thus, N^{17} and polyproline dance partners may keep the Cha-cha-cha-prone polyQ stretch of huntingtin in step, and thereby prevent a toxic conformational stumble.

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Keeping an Eye on Membrane Transport by TR-WAXS

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In this issue of *Structure*, Andersson et al. apply time-resolved wide angle X-ray scattering (TR-WAXS) to follow light-induced conformational changes for both bacteriorhodopsin and proteorhodopsin and probe real-time dynamics at atomic resolution.

Membrane transport proteins perform a multitude of cellular reactions, including energy and signal transduction, regulation of ion concentrations, and transport of metabolites into the cell and noxious substances out. Altered membrane protein function underlies many human diseases, and thus, a deeper understanding of membrane protein structure and dynamics remains a critical objective for basic and medical research. It is well established that membrane transport proteins require distinct temporally regulated structural rearrangements to carry out their biological functions. However, structural details of these dynamic macromolecules have only been studied as snapshots of individual static (and, in most cases, stable) conformations. What is lacking is the ability to capture the transition between these conformations and to probe the role of specific domains and ligands in the process as they proceed through the membrane.

In recent years, our knowledge of membrane protein structure has dramatically increased, providing unforeseen