

# Single Neuron Ubiquitin-Proteasome Dynamics Accompanying Inclusion Body Formation in Huntington Disease\*<sup>§</sup>

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The accumulation of mutant protein in intracellular aggregates is a common feature of neurodegenerative disease. In Huntington disease, mutant huntingtin leads to inclusion body (IB) formation and neuronal toxicity. Impairment of the ubiquitin-proteasome system (UPS) has been implicated in IB formation and Huntington disease pathogenesis. However, IBs form asynchronously in only a subset of cells with mutant huntingtin, and the relationship between IB formation and UPS function has been difficult to elucidate. Here, we applied single-cell longitudinal acquisition and analysis to monitor mutant huntingtin IB formation, UPS function, and neuronal toxicity. We found that proteasome inhibition is toxic to striatal neurons in a dose-dependent fashion. Before IB formation, the UPS is more impaired in neurons that go on to form IBs than in those that do not. After forming IBs, impairment is lower in neurons with IBs than in those without. These findings suggest IBs are a protective cellular response to mutant protein mediated in part by improving intracellular protein degradation.

Huntington disease (HD)<sup>4</sup> is a progressive incurable neurodegenerative disorder caused by the expansion of a polyglutamine (polyQ) stretch in the N-terminal end of the huntingtin (htt) protein above a threshold length of ~36 (1). The deposition of polyQ-expanded aggregated mutant htt in inclusion bodies (IBs) is a hallmark of HD, and IBs are found in human post-mortem samples, transgenic mouse brain, and cell-culture models (2). The accumulation of ubiquitinated proteins in IBs

has implicated the ubiquitin-proteasome system (UPS) in the pathogenesis of HD, amyotrophic lateral sclerosis, Parkinson disease, and polyQ-mediated disorders (3).

The UPS is a major pathway of intracellular protein degradation. After a series of three reactions, each catalyzed by a different set of enzymes, ubiquitin, a 76-amino acid polypeptide, forms an isopeptide bond with the amino group of lysine residues on substrate proteins. Several lysine residues within ubiquitin are sites for more ubiquitin additions. Once a protein accumulates four or more ubiquitins, it is efficiently targeted to the proteasome for degradation. The proteasome binds polyubiquitinated substrates and hydrolyzes ubiquitin isopeptide bonds, releasing ubiquitin moieties before degrading substrate proteins through chymotrypsin-like, trypsin-like, and post-glutamyl peptidase activities (3).

Increased polyubiquitin levels and changes in ubiquitin linkages accompany the accumulation of UPS substrates in the brains of HD patients and transgenic mice and in cellular HD models (4). UPS substrates accumulate throughout the cell in polyQ models, even before IB formation (5, 6). This has added to the confusion over whether polyQ expansion leads to toxicity through direct impairment of proteasomal degradation. Proteasomes have been reported to cleave polyQ stretches efficiently (7), inefficiently (8), or essentially not at all (9). *In vivo*, polyQ-dependent degeneration occurs with no detectable proteasome inhibition (10, 11) or is tightly linked to it (12, 13). The inability of some studies to detect UPS impairment in HD models may be due to the limited sensitivity of conventional approaches to identify cell-to-cell variations in UPS function.

The relationship between IB formation and UPS function has been difficult to determine. Protein turnover in cells with IBs is evidently reduced and accompanied by the accumulation of cellular proteins (14–16); HEK293 cells containing mutant htt IBs have a greater degree of UPS impairment than those without IBs (5). Proteasome subunits and heat shock proteins colocalize with IBs, but it is unclear if this colocalization facilitates protein delivery or unfolding at the mouth of active proteasomes, or if it harms proteasome function by sequestering essential cellular machinery (18). Some IBs are relatively static (8, 25), but the proteins in others are dynamically exchanged with cytoplasmic and nuclear pools (19, 20).

UPS function is critical to cellular homeostasis. Deletion of one of the two inducible polyubiquitin genes in mice leads to lower intracellular ubiquitin levels in germ cells and hypothal-

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<sup>4</sup> The abbreviations used are: HD, Huntington Disease; UPS, ubiquitin-proteasome system; IB, inclusion body.

lamic neurons. These same populations undergo cell-cycle arrest and hypothalamic neurodegeneration, respectively (22, 23). Cell lines expressing mutant huntingtin accumulate ubiquitinated proteins and undergo cell-cycle arrest in G2/M (5). In neurons, UPS impairment may lead to cell death through an accumulation of signals for apoptosis, a decrease in NF- $\kappa$ B signaling, sensitization to other toxic stimuli, remodeling of synapses, retraction of neurites, or other unidentified mechanisms (24). The effect of UPS impairment depends on cell type and cell cycle, and the relationship between UPS impairment and striatal neuronal survival is largely unknown.

Diffuse species of mutant htt induce IB formation and neuronal death in a protein concentration-dependent manner (2). IB formation delays neuronal death, suggesting that IB formation helps neurons cope with toxic diffuse mutant htt. Whether the effect of IB formation on survival is mediated through UPS function has been difficult to determine. IB formation and neuronal death occur asynchronously in overlapping but distinct subsets of neurons that express mutant htt. The observation that IB formation is not required for UPS impairment also complicates population analysis (6, 26).

To explore this problem, we applied single-cell analysis. We tracked single neurons over their entire lifetimes, gaining spatial and temporal resolution while simultaneously monitoring IB formation, UPS inhibition, and neuronal toxicity.

## EXPERIMENTAL PROCEDURES

**Plasmids**—mRFP (27), pCS2-Venus (28), and pEGFP-CL1(5), pGW1-GFP, pGW1-httQ<sub>72</sub>-eGFP, pGW1-mRFP (2) have been described. pGW1-httQ<sub>72</sub>-CFP was generated from pGW1-httQ<sub>72</sub>-eGFP. pGW1-mRFP<sup>u</sup> (mRFP<sup>u</sup>) was generated by subcloning mRFP1 from pcDNA3.1(+) into pEGFP-CL1; mRFP1-CL1 was then subcloned into pGW1. pGW1-GFP<sup>u</sup> was constructed by excising EGFP-CL1 from pEGFP-CL1 and inserting it into pGW1. pGW1-Venus-CL1 (Venus<sup>u</sup>) was generated by subcloning Venus from pCS2-Venus into pcDNA3.1(+). The stop codon from Venus was removed and replaced by the sequence AGATCTCG. The CL1 sequence (5) was introduced at the 3'-end of Venus. Venus-CL1 was then subcloned into pGW1. pCS2-Ub<sup>G76V</sup>-Venus (Ub<sup>G76V</sup>-Venus) was generated by PCR of Ub<sup>G76V</sup> from Ub<sup>G76V</sup>-GFP (29).

**Cell Culture**—Striata from rat embryos (E17–18) were dissected, dissociated, and plated on 24-well tissue-culture plates ( $5.8 \times 10^5$ /well) coated with poly-D-lysine and laminin (BD Biosciences, San Jose, CA) as described (2, 30). The cells were grown in 1 ml of modified neuronal culture medium (NCM). Cells were fed every 5–7 days by replacement with equal measures of conditioned and fresh neuronal culture medium.

**Transfection, Pharmacology, and Colocalization**—Primary cultures were transfected 5–7 days *in vitro* with combinations of pGW1-GFP<sup>u</sup> and pGW1-mRFP, pGW1-mRFP<sup>u</sup>, and pGW1-GFP, pGW1-Venus<sup>u</sup>, or pCS2-Ub<sup>G76V</sup>-Venus, and pGW1-CFP, and pGW1-httQ<sub>72</sub>-eGFP, pGW1-mRFP<sup>u</sup>, and pGW1-BFP, or pGW1-httQ<sub>72</sub>-CFP and pYFP-LMP2 in a 1:1 or 1:1:1 molar ratio with 2–4  $\mu$ g of total plasmid DNA per well. Our transfection protocol was described (2). MG132 (Sigma-Aldrich), epoxomicin (Boston Biochem, Cambridge, MA), and Bafilomycin A1

(Sigma-Aldrich) were added in 1 ml of conditioned NCM per well 12–60 h after transfection.

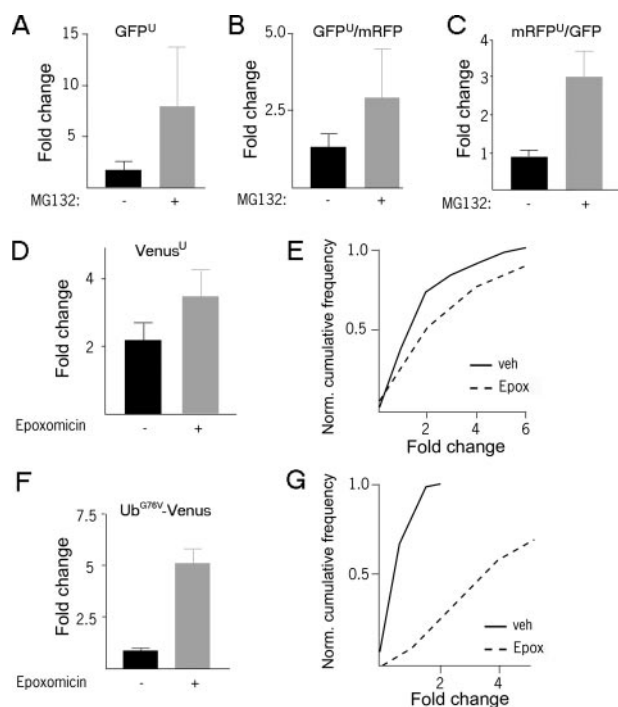
Colocalization of fluorescence was calculated using Metamorph. Briefly, images of fluorescence from CFP-htt, ubiquitin staining, or LMP-YFP were analyzed, and pixels were classified as “positive” if their intensity was 3 $\times$  greater than background pixels. The fraction of positive pixels for CFP-htt IBs that overlapped positive pixels of ubiquitin staining or LMP-YFP fluorescence was calculated with Metamorph for ( $n = 20$  neurons).

**Live-Cell Imaging and Analysis**—Images of cells were obtained with a robotic microscope system as described (2, 32). Briefly, the imaging was performed with a Nikon TE300 inverted microscope with a long working-distance Nikon 20 $\times$  (NA 0.45) objective. Stage movements and focusing were performed using a Proscan II stage controller (Prior Scientific, Rockland, MA). Samples were illuminated with a 175 watt Xenon Lambda LS illuminator (Sutter Instruments, Novato, CA). Blue, green, and red fluorescent protein (BFP, GFP, and RFP, respectively) images were captured using an 86014 beamsplitter and 350/50 $\times$ ; 465/30m, 480/40 $\times$ ; 517/30m and 580/20 $\times$ ; 630/60m fluorescence filters respectively. CFP, Venus, and RFP images were captured using a 86006 beamsplitter and 420/35 $\times$ ; 470/30m, 500/20 $\times$ ; 535/30m, and 580/20 $\times$ ; 630/60m fluorescence filters (Chroma Corp, Rockingham, VT). Algorithms for plate registration, stage movements, filter movements, focusing, and acquisition were generated with Metamorph imaging software (Molecular Devices, Sunnyvale, CA). Images were analyzed manually using Metamorph software. Fully automated acquisition and analysis algorithms have been created (Media Cybernetics, Bethesda, MD). Survival analysis was performed with the Statview software package (SAS Institute, Cary, NC); *t* tests for comparisons of means and two-sample Kolmogorov-Smirnov tests for comparisons of distributions were performed with Prism (Graphpad Software, San Diego, CA).

## RESULTS

**Longitudinal Live-Cell Monitoring of UPS Function in Primary Neurons**—To monitor dynamic changes in protein degradation in live cells, we used a unique high-throughput image acquisition platform (2, 32) and fluorescent protein substrates of UPS degradation. We used fluorescent proteins with the CL1 peptide fused to the C terminus (34) or a non-hydrolyzable ubiquitin moiety (Ub<sup>G76V</sup>) fused to the N terminus (35) to target them to the UPS for degradation. These destabilized fluorescent proteins were transfected into primary neurons and fluorescence in individual cells was monitored for hours or days to detect changes in the degradation of UPS substrates. To control for nonspecific changes in transcription and protein handling while monitoring cell survival (2), we co-transfected and tracked the fluorescence of unmodified fluorescent proteins in the same cells.

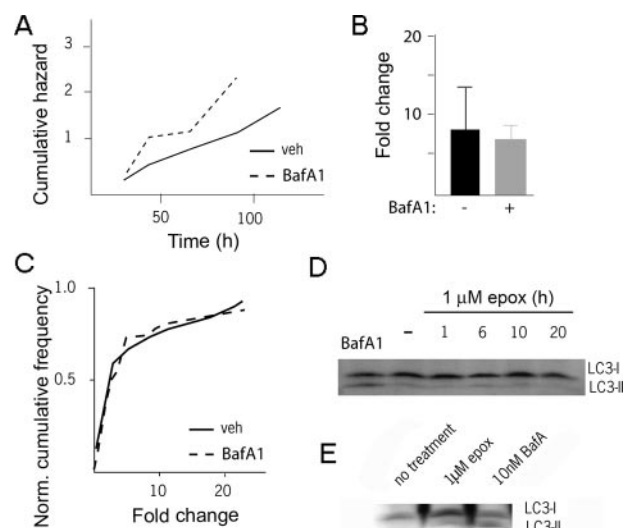
**Destabilized Fluorescent Proteins Accumulate after Proteasome Inhibition in Primary Neurons**—Fluorescence intensity in live cells is an accurate indicator of the amount of fluorescent protein within the cell (2). Fluorescence levels in primary striatal neurons of a destabilized form of enhanced GFP<sup>u</sup> (5) (Fig. 1A), monomeric mRFP<sup>u</sup> (27) (Fig. 1C), or two forms of the



**FIGURE 1. Levels of proteasome reporters increase after inhibition of proteasome.** *A*, after transfection with GFP<sup>U</sup> and mRFP, striatal neurons were treated with 50  $\mu$ M MG132 for 12 h. GFP fluorescence (*A*) and the ratio of GFP<sup>U</sup>/mRFP fluorescence (*B*) both increase after treatment relative to control. *C*, after transfection with mRFP<sup>U</sup> and GFP, striatal neurons were treated with 50  $\mu$ M MG132 for 12 h. The mRFP<sup>U</sup>/GFP ratio is significantly greater than the control ( $p < 0.02$ ). *D*, after transfection with Venus<sup>U</sup> and CFP, striatal neurons were treated with 2  $\mu$ M epoxomicin (solid lines) or vehicle (broken lines) for 10 h. Both mean change in Venus<sup>U</sup>/CFP fluorescence (*D*) and single-cell distributions of Venus<sup>U</sup>/CFP fluorescence (*E*) are increased relative to control ( $p < 0.05$ ,  $p < 0.05$ ). *F*, after transfection with Ub<sup>G76V</sup>-Venus and CFP, striatal neurons were treated with 2  $\mu$ M epoxomicin for 10 h. Both mean change in Ub<sup>G76V</sup>-Venus/CFP fluorescence (*F*) and single-cell distributions of Ub<sup>G76V</sup>-Venus/CFP fluorescence (*G*) are increased ( $p < 0.05$ ,  $p < 0.01$ ). Experiments were repeated twice with over 50 cells analyzed in each condition.

enhanced yellow fluorescent protein variant Venus (Ub<sup>G76V</sup>-Venus and Venus<sup>U</sup>) (28) (Fig. 1, *D–G*) increased after treatment with proteasome inhibitor, even when changes in fluorescence of unmodified spectrally distinct fluorescent proteins in the same cells was controlled for (Fig. 1, *B, C, E, G*). The significant and rapid increase in fluorescence of these reporters from low baseline levels after proteasome inhibition in primary neurons is in agreement with previous work in cell lines (5, 6, 26). Addition of the CL1 peptide or Ub<sup>G76V</sup> degron to fluorescent proteins did not cause the proteins to aggregate when they were expressed in neurons, unlike observations from cell lines (36).

**Inhibiting Autophagy Does Not Result in Accumulation of UPS Reporters in Primary Neurons**—To ensure that these destabilized proteins were targeted primarily to the UPS for degradation, we used Bafilomycin A1 (BafA1) to inhibit autophagy. BafA1, a vacuolar ATPase inhibitor, prevents autophagosome-lysosome fusion and causes the accumulation of substrates targeted for macroautophagy (37). BafA1 caused a rapid accumulation of the membrane-bound form of microtubule-associated protein 1 light chain 3 (LC3-II) and was toxic to primary neurons (Fig. 2, *A* and *D*), but BafA1 did not increase levels of UPS reporters (Fig. 2, *B* and *C*).

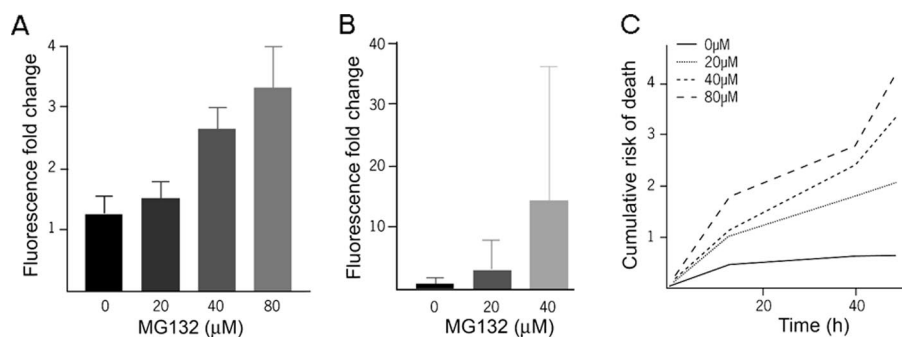


**FIGURE 2. Limited interaction between the UPS and autophagic pathways in neurons.** *A*, 24 h after cotransfection with Ub<sup>G76V</sup>-Venus and CFP, striatal neurons were treated with vehicle or 50 nM BafA1. BafA1 treatment caused a significant amount of toxicity above control ( $p < 0.03$ , top line). Mean Ub<sup>G76V</sup>-Venus/CFP ratio (*B*) and the distribution of the single-cell changes in Ub<sup>G76V</sup>-Venus/CFP (*C*) in these cells did not increase above control in 20 h after BafA1 addition. *D*, neurons or HEK293 cells (*E*) were treated with BafA1 or epoxomicin, followed by Western blotting with an LC3 antibody. While BafA1 caused accumulation of LC3-II in both neurons and HEK293 cells, epoxomicin increased LC3-II levels only in HEK293 cells. Unlabeled lanes in *E* are lysates from cells transfected with LC3.

**Proteasome Inhibition Does Not Change LC3-II Levels in Primary Neurons**—Proteasome inhibition can increase flux through the autophagic pathway in some cells (13). To determine if autophagic activity could be confounding fluorescent reporter measurements of UPS function, we examined the activity of the autophagic pathway after proteasome inhibition. The level of LC3-II is commonly used as a surrogate for the number of autophagosomes and flux through the macroautophagic pathway. After treatment with epoxomicin, primary neurons showed no change in LC3-II levels (Fig. 2*D*), though as seen in previous reports, LC3-II accumulated in HEK293 cells (Fig. 2*E*).

**UPS Reporter Fluorescence Demonstrates a Graded Response to Proteasome Inhibition**—Having validated the use of destabilized fluorescent proteins as reporters of UPS function in primary neurons, we then examined the nature of their response to varying levels of proteasome impairment. We co-transfected mRFP<sup>U</sup> and GFP into primary striatal neurons and treated the cells with increasing doses of the proteasome inhibitor MG132. Though fluorescent UPS reporters have been reported to relocalize to IBs, we found that mRFP<sup>U</sup> fluorescence remained diffuse in striatal neurons after proteasome inhibitor treatment (6). As early as 2.5 h after addition of MG132, reporter fluorescence increased in proportion to the amount of MG132 added (Fig. 3*A*), and reporter fluorescence continued to increase over time (Fig. 3*B*). Thus, in primary neurons, the increase in fluorescence of these proteins faithfully reports the extent of proteasome impairment (5, 6).

By monitoring individual cells treated with MG132 over days, we determined the effect of increasing proteasome inhibition on the survival of primary striatal neurons. When the dose of MG132 increased, neurons died faster (Fig. 3*C*). These



**FIGURE 3. Inhibition of proteasome activity is toxic in a dose-dependent fashion.** *A*, UPS reporter fluorescence shows a dose-dependent response to MG132 treatment. MG132 at the indicated doses was added to striatal neurons 24 h after transfection with mRFP<sup>u</sup> and GFP. The change in mRFP<sup>u</sup>/GFP ratio over the first 2.5 h after MG132 administration is shown. *B*, UPS reporter fluorescence continues to increase up to 12 h after the addition of MG132. Note the difference in scale with *A*. Measurements from 80 μM were excluded due to noticeable toxicity. *C*, MG132 is toxic to neurons in a dose-dependent fashion. The same neurons shown in *A* and *B* were observed with the risk of death as shown. Longitudinal analysis was repeated twice on different transfections, with  $n > 50$  for each treatment in each experiment.

neurons demonstrated a proportional relationship between proteasome impairment and the accumulation of UPS substrates; similarly, there was a proportional relationship between proteasome impairment and neuronal toxicity.

**Longitudinal Live Cell Detection of UPS Function in a Primary Neuronal Model of HD**—We then examined a primary striatal model of HD (2, 30) and prospectively followed visual markers of UPS function, IB formation, and neuronal viability in single cells. This model reproduces key features of HD, including neuronal subtype specificity (30) and polyQ length-dependent toxicity (2, 30). To induce the HD disease phenotype in this model, we transiently transfected an N-terminal htt fragment with 72 glutamines fused to GFP (htt<sup>ex1</sup>-Q<sub>72</sub>-GFP). We simultaneously introduced mRFP<sup>u</sup> and BFP into the same neurons to monitor UPS impairment and cell viability, respectively. Virtually all IBs in this model stain with ubiquitin and colocalize with proteasome subunits (supplemental Fig. S1). From series of images of individual neurons, we quantified single-cell changes in UPS reporter fluorescence over the lifetimes of cells expressing the htt<sup>ex1</sup>-Q<sub>72</sub>-GFP protein (Fig. 4A).

Would UPS function differ in neurons that do and do not form IBs? By reviewing images from our longitudinal analysis experiments, we identified neurons that had or had not formed an IB at some point over the course of the experiment. From these two groups, we then chose neurons that were from the same well of the culture dish to form two cohorts based on IB formation. To reduce potential biases introduced by using IB formation as a selection criterion, we included only neurons that had already lived the same length of time *in vitro*. We then monitored UPS reporter fluorescence in neurons before, during, and after IB formation and compared it to that in the cohort of age-matched neurons that did not form IBs.

**UPS Impairment Precedes IB Formation**—Those cells that would go on to form IBs had significantly larger increases in UPS reporter fluorescence before IB formation, both in the single-cell distribution of reporter fluorescence (Fig. 4B) and in mean reporter fluorescence (Fig. 4C). This relationship was independent of the time at which IBs formed (Fig. 4D).

**UPS Impairment Decreases after IB Formation**—To determine if IB formation improves or worsens UPS function, we examined UPS reporter fluorescence in neurons during and after IB formation. We compared these measurements to UPS reporter fluorescence in an otherwise matched cohort of neurons that did not form IBs over the same interval. To again reduce potential biases introduced by using IB formation as a selection criterion, neurons from each cohort were matched for the length of time they lived *in vitro*. We found that neurons that formed IBs had significantly smaller increases in UPS reporter fluorescence (Fig. 4, E and

F), indicating that less UPS impairment occurs in cells after IB formation than in cells that did not form IBs.

**IB Formation Improves Neuronal Survival**—To determine if this reduced UPS impairment changes neuronal survival, we compared the survival of neurons that we analyzed for UPS function. When we examined matched cohorts of neurons transfected with htt<sup>ex1</sup>-Q<sub>72</sub>-GFP, mRFP<sup>u</sup>, and BFP that formed or did not form IBs, those cells that formed IBs survived longer (Fig. 4, G and H). This finding agrees with previous results showing that neurons survive longer if they form IBs (2).

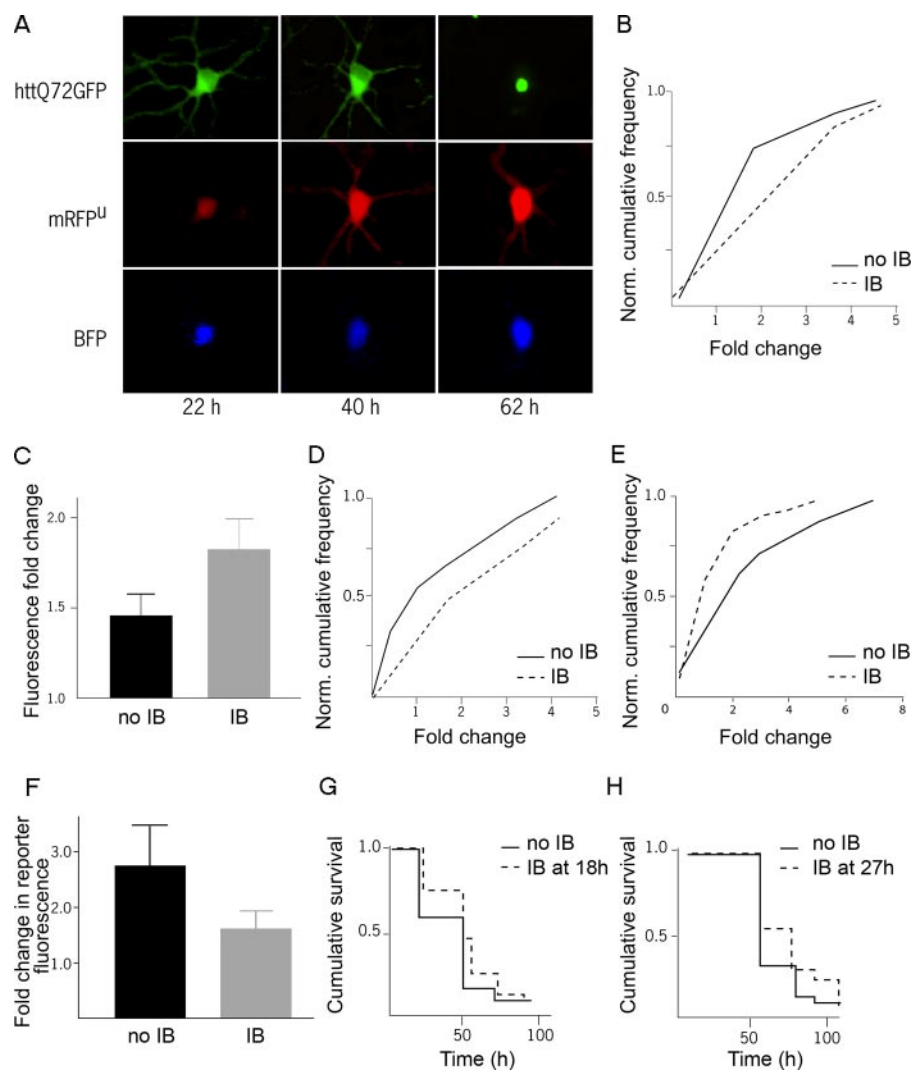
## DISCUSSION

By applying a high-throughput single-cell longitudinal imaging platform to a neuronal model, we were able to examine the events in the cellular pathogenesis of HD with improved sensitivity and temporal resolution. Through the use of spectrally distinct fluorescent species, we simultaneously monitored neuronal viability, htt IB formation, and intracellular protein degradation. We found that neurons that form IBs have increased UPS impairment preceding IB formation and less UPS impairment after IB formation than cells that do not form IBs. Though tonic UPS inhibition is toxic to primary striatal neurons, neurons that formed IBs survived better than those that did not. These results support a model in which IB formation reflects a beneficial cellular response to mutant protein, mediated in part by restoring UPS function.

Though multiple pathways of intracellular protein degradation may handle aggregation-prone protein, we found that some proteins are likely targeted primarily to the UPS for degradation. In our experience with fluorescent UPS reporters, we found little evidence that they are routinely degraded by autophagy. Though it is clear that autophagy modulates the turnover and toxicity of aggregation-prone proteins, the addition of the CL1 or Ub<sup>G76V</sup> degron does not cause fluorescent proteins to aggregate in neurons. This discrepancy with other reports in cell lines may be due to lower expression levels in neurons after transient transfection.

The finding that proteasome inhibition is not sufficient to change the flux through the autophagic pathway in primary

## Ubiquitin-Proteasome Dynamics in Huntington Disease



**FIGURE 4. IB formation and UPS function in primary neurons.** *A*, GFP-htt, BFP, and mRFP<sup>u</sup> were imaged over the course of days to follow htt IB formation, UPS impairment, and neuronal survival. Single-cell distributions (*B*) or population means (*C*) of the change in mRFP<sup>u</sup>/GFP fluorescence in the interval preceding IB formation at 54 h. The increase in mRFP<sup>u</sup>/GFP ratio was higher in neurons that went on to form IBs ( $p < 0.05$ ,  $p < 0.05$ ). *D*, in a parallel experiment, single-cell distributions of the change in mRFP<sup>u</sup>/GFP fluorescence in the interval preceding IB formation at 76 h also show higher UPS impairment in those neurons that will go on to form IBs ( $p < 0.05$ ). After 54 h, single-cell distributions (*E*) or population means (*F*) show a greater increase in mRFP<sup>u</sup>/GFP fluorescence in those cells that did not form IBs ( $p < 0.05$ ,  $p < 0.05$ ). The survival of those neurons that formed htt IBs at 18 h (*G*) or 27 h (*H*) was better than the survival of neurons that survived at least that long but never formed IBs ( $p < 0.01$ ,  $p < 0.03$ ). Longitudinal analysis was repeated twice in different experiments with over 300 cells analyzed in each experiment, with  $n > 30$  for each cohort.

neurons also highlights possible differences between mammalian neurons and other model systems. The difference in behavior of the autophagic pathway in mammalian neurons may be due to a difference in constitutive activity (39). While most non-neuronal cells upregulate autophagy after 24 h of starvation, neurons do not *in vivo* (40) or *in vitro*<sup>5</sup> even after longer starvation periods. The finding that the deletion of essential autophagic machinery results in a neurodegenerative phenotype points to a critical role in neuronal function and survival (38, 41).

Though it remains unclear how IB formation is functionally linked to an improvement in UPS function, one possibility is that IB formation is a step toward shunting aggregation-prone

proteins normally targeted to the UPS to other pathways of intracellular protein degradation. In both yeast and mammalian cells, misfolded and aggregation-prone proteins may be targeted to different intracellular compartments depending on the availability of ubiquitin (31). Differential localization may be one component of targeting proteins to the autophagic pathway of protein degradation, which has been implicated in the clearance of aggregation-prone protein, including mutant htt (17, 21). If expanded polyQ tracts impair the ability of the proteasome to degrade other cellular proteins (9) or if ubiquitination is inadequate due to ubiquitin sequestration by IBs, shifting polyQ degradation from the UPS to the autophagic pathway could effectively increase the flux of other proteins through the UPS.

A second possibility that is not mutually exclusive is that IB formation is part of a cellular program to more efficiently degrade protein through the UPS. The recruitment of chaperones and proteasomal machinery to intracellular inclusions varies based on protein and cell type (19, 25). Though the IBs in our primary neuronal model are long-lived, with fewer than 2% disappearing before the neuron that contains them dies (2), a small proportion of cells can clear IBs, and a detailed longitudinal analysis of these cells will likely be informative.

Previous work suggested that IB formation safely sequesters more toxic forms of mutant htt to improve neuronal survival. This study suggests two additional mechanisms by which IB formation might contribute to improved cell survival after IB formation. First, we found that tonic UPS inhibition is toxic and that IB formation is associated with a relative improvement in UPS function. Thus, IB formation may partially restore longevity by improving UPS throughput and consequently lowering the overall cellular burden of misfolded proteins. A second but related possibility is suggested by reports that transient sublethal proteasome inhibition can induce cells to adapt in ways that protect them against further insults (33). Transient proteasome inhibition might trigger a cell-wide adaptive response in neurons that may involve coordinated changes in molecular chaperones and protein turnover pathways. If so, such an adaptive response may be important in a variety of neurodegen-

<sup>5</sup> A. Tsvetkov and S. Finkbeiner, unpublished observations.

erative diseases that result from misfolded intracellular proteins.

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