JCB Journal of Cell Biology

SPOTLIGHT

# Endosomal recycling defects link Huntington's disease with McLeod syndrome

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**Chhetri and colleagues (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202112073) show that Rab11-mediated endosomal recycling regulates cell surface expression of McLeod syndrome protein XK. Mutant huntingtin interferes with the recycling of XK to the cell surface and significantly reduces manganese transport across cell membrane.**

Huntington's disease (HD) is an autosomal dominant disease caused by a CAG trinucleotide repeat expansion in exon 1 of the huntingtin (*HTT*) gene. Patients with *HTT* mutations present with choreiform movement dysfunction, psychiatric changes, and cognitive impairment. Neurodegeneration in HD preferentially affects the medium spiny neurons of the striatum, with subsequent involvement in cortical neurons and other brain regions as disease progresses (1). WT HTT protein is a ubiquitous protein in brain and contains multiple functional domains that can affect diverse biological processes, including transcription, mitochondria, endoplasmic reticulum, and lysosomes, which can be targeted by mutant HTT (mHTT) proteins to promote pathogenesis of HD. Interestingly, several proteins have been shown to interact with, and modify the functions of, HTT. Some of these HTT interacting partners show selective expression in the striatum, thus offering insights that could help explain why the striatal neurons are selectively vulnerable in HD.

Reporting in this issue of *JCB*, Chhetri and colleagues (2) used sucrose gradient-based subcellular fractionation and affinity chromatography to enrich Rab11-containing endosomal vesicles from neural progenitors from STHdhQ7/Q7 and STHdhQ111/Q111 transgenic mice (2). The rationale was based on the role of Rab11 as a critical member of small GTPase that regulates endosome recycling to the cell surface, which has been shown to be critical for redirecting proteins, such as receptors, transporter, and adhesion molecules (3). Indeed, using quantitative proteomics the authors identified proteins with these functional properties that were more enriched in STHdhQ111/Q111. Interestingly, one of the HTT interacting partners is XK, which is a subunit of the Kell Blood Group Complex and an endoplasmic reticulum membrane adapter protein. Interestingly, mutations in XK are causally linked to McLeod neuroacanthocytosis syndrome, an X-linked recessive disorder characterized by abnormal star-shaped red blood cells and involuntary movements, including chorea and dystonia (4). The connection between XK and HTT is particularly intriguing because movement disorders are prominent clinical features of in both Huntington's disease and McLeod syndrome. Furthermore, several previous studies have implicated Rab11 as a modifier of mHTT functions. Together, these results provide intriguing clues to further delineate the role of XK and Rab11 in the pathogenesis of HD.

To further investigate how XK affects Rab11-mediated endocytosis, the authors generated a chimeric XK-EGFP protein by inserting EGFP into the N-terminus of XK protein, just after the signal peptide. By coexpressing XK-EGFP with mCherry-labeled vesicles markers, such as Rab4, Rab5, and Rab11, the authors confirmed that XK was indeed associated with Rab11$^+$ in the recycling endosomes. To assess if the XK protein is a cargo of Rab11$^+$ in the recycling endosomes, the authors performed immunofluorescent microscopy in live cells, cell surface biotinylation, and Western blots to show that in WT STHdhQ7/Q7 striatal cells expressing dominant negative Rab11 mutant reduced XK on the cell surface (Fig. 1). In contrast, dominant active Rab11 (dArab11) increased XK on the cell surface. These results support that Rab11 regulates XK trafficking to cell surfaces. Next, the authors interrogated the effect of mHTT on XK localization and transport using live cell imaging on cells co-expressing XK-EGFP and mCherry-Rab11. In mouse striatal neurons expressing STHdhQ111/Q111 mHTT, fewer small motile vesicles containing both XK-EGFP and mCherry-Rab11 were detected, and there were less dynamic large tubulovesicular structures compare to WT cells, suggesting that XK trafficking is impaired in HD striatal cells. Interestingly, co-expression of dArab11 in STHdhQ111/Q111 HD cells rescued the XK trafficking defects, suggesting that XK trafficking is due to the Rab11 activity deficiency in HD cells. Using a pH-sensitive pHluorin and cell surface-labeling using XK antibody, the authors

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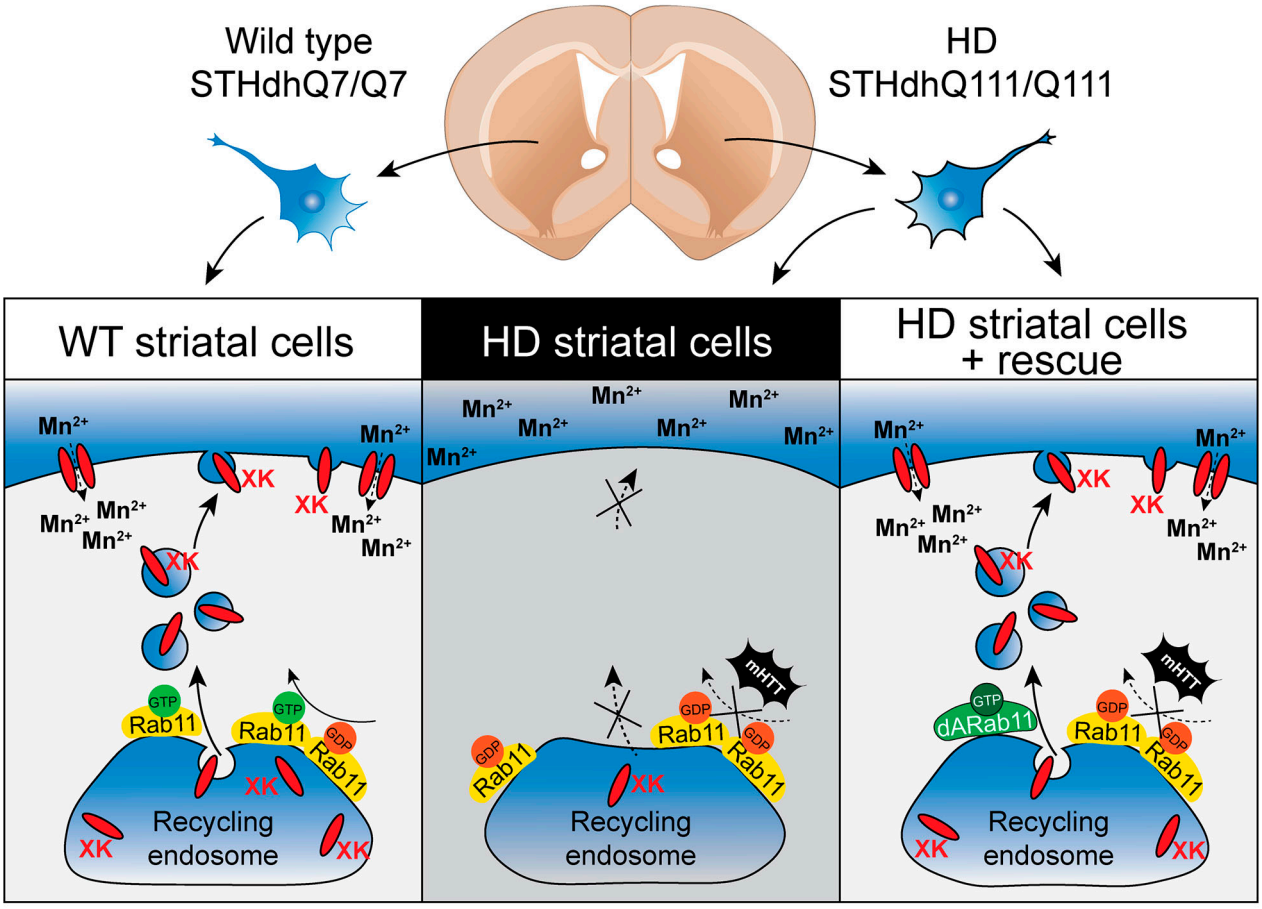


Figure 1. **Rab11-mediated endosomal recycling defects reduce cell surface expression of McLeod syndrome protein XK and Mn transport in striatal neurons in HD.**

found that XK expression was drastically decreased on the cell surface in STHdhQ111/Q111 HD cells compared to WT cells. Consistent with these results, immunofluorescent microscopy confirmed reduced XK expression on the surfaces of striatal cells in the adult brain of HD140Q/140Q mice.

Finally, to provide more insights into the role of XK in HD pathogenesis, the authors asked how reduced cell surface expression of XK affected the cellular homeostasis of manganese (Mn), which is a documented function of XK in red blood cells (5). In addition, Mn is most abundantly detected in the striatum (6), and reduced Mn has been reported in the postmortem brain tissues in HD patients (7), and in STHdhQ111/Q111 cells and YAC128 HD mice (8–10). To this end, the authors used in silico modeling to identify potential Mn-binding sites in XK, and molecular dynamic simulation showed that, upon binding with Mn, XK underwent conformational changes that suggested XK as a potential Mn transporter on the cell surface. These results were further validated using Fura-2 fluorescent quenching assays to show that STHdhQ111/Q111 HD cells contained significantly reduced Mn levels, which can be restored by expressing dArab11.

Taken together, this study provides an integrated view as to how perturbations to Rab11-mediated recycling of XK to the cell surface of striatal cells could lead to an imbalance in Mn import and thereby increases neuronal vulnerability in the striatum of patients with HD (Fig. 1). While the results in this study offer exciting new insights into HD pathogenesis, there are many unanswered questions. For instance, it remains unclear how mHTT affects endosomal trafficking and recycling of membrane transporters, such as XK, to the cell surface. In addition to XK, what other membrane-bound receptors or transporters might be affected by this endosomal trafficking defects? Finally, the impacts of mHTT on endosomal trafficking may capture a small part of a broader role of mHTT in disrupting endolysosomal trafficking, which has been shown to contribute to other neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, frontotemporal lobar degeneration, and amyotrophic lateral sclerosis. Future studies will bring additional insights to intracellular trafficking pathways that promote neurodegeneration in disease-specific manners.

## Acknowledgments

Funding provided by postdoctoral fellowship from the American Federation for Aging Research and Glenn Foundation for Medical Research (E. Marsan), National Institutes of Health grants R01 AA027074, R01 AG057462, and R01 AG068290, and VA Merit Review Award I01 BX001108 (E.J. Huang).

The authors declare no competing financial interests.

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