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Spotlight

Lysosomes nor Mice Move Forward without Borcs7

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Lysosome function and position in the cytoplasm depends on the BORCS machinery, which tethers lysosomes to the kinesin microtubule motor. A recent paper of Snouwaert *et al*. in *Cell Reports* characterizes a mouse with a spontaneous mutation in the Borcs7 subunit, which causes axonal dystrophy and impaired motor function.

Lysosomes are a heterogeneous class of dynamic and versatile organelles that represent the main degradative compartment of the endomembrane system in metazoan cells. Lysosomes were long perceived to be merely the final destination of endocytosed biomolecules and cellular waste that is cleared by autophagy. The ability to analyze them in individual cells with improved microscope techniques and immunological and genetic reagents, however, revealed a far more functional complex repertoire. This includes, among others, roles in wound healing, cellular cytotoxicity, antigen presentation, cell migration, and nutrient signaling [1].

Lysosome distribution is typically bimodal, with a clustered pattern in the region surrounding the microtubule-organizing center where organelles of the endomembrane system are concentrated and a second population that is broadly dispersed throughout the cytoplasm. The two populations have different molecular make-ups reflecting their distinct properties. Movement of peripheral lysosomes to the cell center is accompanied by their maturation to a state featuring a more acidic lumenal pH and higher degradative capacity [2]. Lysosomes move bidirectionally in a saltatory modality along microtubules [3]. Dynamic interactions of lysosomes with, and the net outcome of motility driven by, kinesin and cytoplasmic dynein microtubule motor proteins largely determine this dynamic partitioning to either of the two pools [2]. Additional cellular processes that control positioning of lysosomes are their transient association with the endoplasmic reticulum (ER) in ER–lysosome contact sites [2] and through coordinated activity of microtubule- and actin-based motility as in the periphery of melanocytes and cytotoxic T cells.

The molecular principles underlying bidirectional lysosomal motility are fairly well understood. Centripetal transport towards the cell centre is regulated by rab7. The GTP form of this small GTPase in association with the effector RILP recruits the minus end-directed dynein–dynactin microtubule motor complex onto lysosomes. Centrifugal transport to the cell periphery is regulated by the lysosome-associated hetero-octameric BLOC-1-related complex (BORC) (Figure 1A). BORC comprises five unique subunits and shares the BLOS-1, BLOS-2, and snapin subunits with the BLOC-1 complex that has been implicated in minus end-directed motility [2]. BORC may serve as an exchange factor of Arl8 [4] and is required for recruitment of the small GTPase to lysosomes. Arl8 binds either directly to a plus end-directed motor (as for KIF5B) [2,6] or via the scaffold SKIP for KIF1 [5,6] (Figure 1B).

Long-distance transport of lysosomes is vital in highly polarized neurons where waste products of constitutive autophagy generated at the distal tip of the axon are degraded in the cell body [7]. Deletion of BORC in primary hippocampal neurons affects autophagosome turnover and growth cone dynamics [6]; a role for BORC-dependent lysosome transport *in vivo*, however, has not yet been established. A recent paper of Snouwaert *et al*. in *Cell Reports* [8] now shows that a spontaneous missense mutation in the Borcs7 subunit of BORC causes progressive axonal dystrophy with severe loss of motor function, a phenotype with similarities to human hereditary spastic paraplegia. The mutation, named Borcs7-Q87X, causes a truncation of 18 amino acids at the carboxy terminus.

The mice were originally identified because of their altered gait and by hind-limb clasping on lifting by the tail, the latter of which is a proxy for disease progression during neurodegeneration as a consequence of impaired autophagy in mice. Comparative analysis of behavior and motor defects in affected animals showed that the mutant mice were most severely challenged in motor tasks dependent on rear limb function. Histological evaluation of lumbar spinal cord sections from mutant mice revealed swollen spherical bodies often containing organelles in several stages of degeneration and swollen axons, indicative of dystrophy of both motor and sensory tracks. These morphological abnormalities reflect pathological phenotypes seen in axonopathies associated with neuroaxonal dystrophy and hereditary spastic paraplegia in rat and are suggestive of a malfunctioning lysosome–autophagy pathway [8].

mRNA analysis showed that Borcs7 is highly expressed in both the olfactory bulb and the frontal cortex, where it is found in glial cells and neurons. Remarkably, the highest Borcs7 levels were detected in macrophages, suggesting that Borcs7 does not act exclusively in

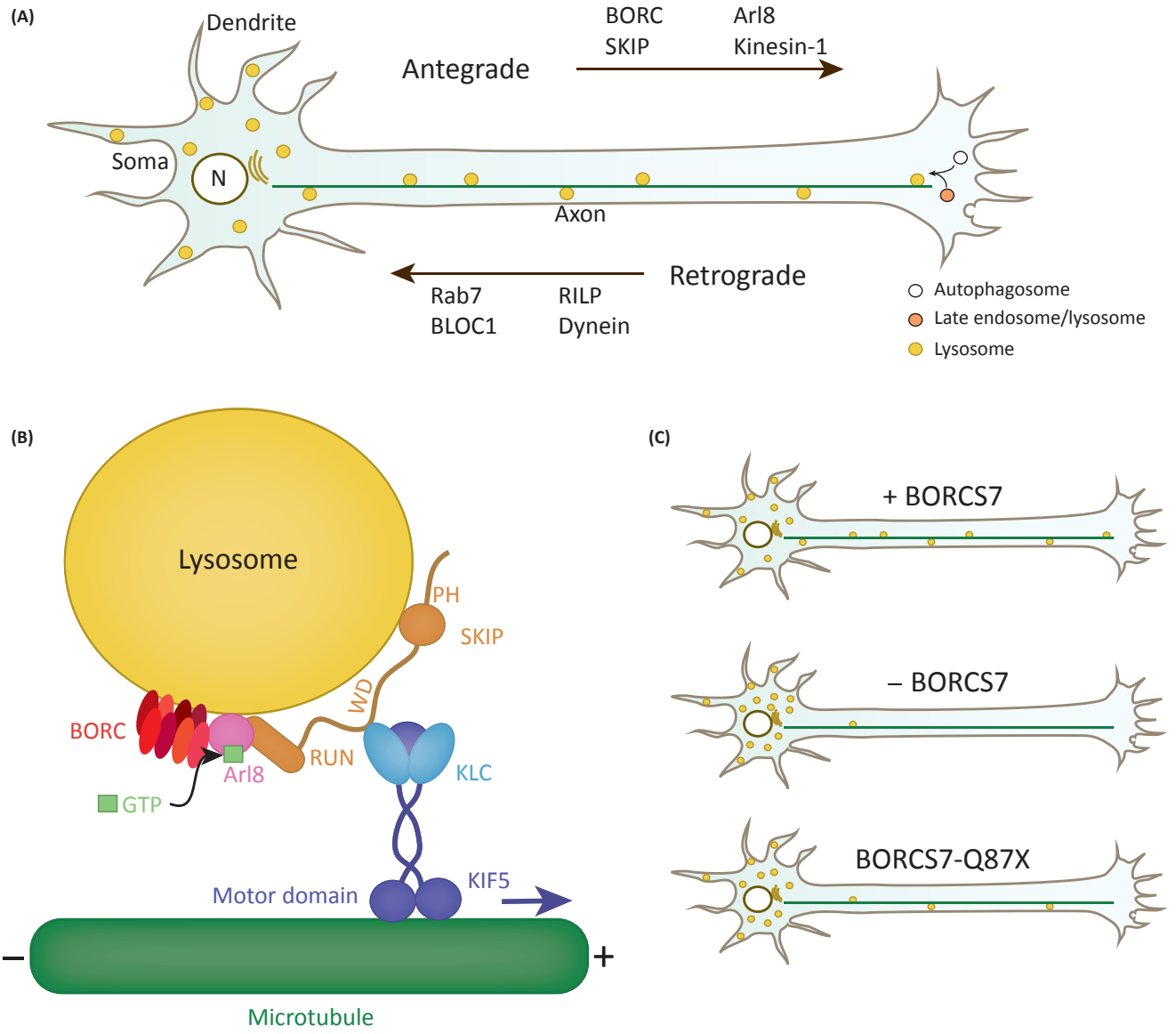

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Figure 1. Transport of Lysosomes along Microtubules. (A) Diagram outlining + end (antegrade) and – end (retrograde) transport of lysosomes and lysosomes that are fused with autophagosomes at the distal end of the axon from and to the cell body, respectively. Key players in antegrade transport include BLOC-1-related complex (BORC), Arl8, SKIP, and kinesin-1, while for retrograde motility rab7, its effector RILP, BLOC-1, and the dynein microtubule motor are used. (B) Model for the role of BORC in plus end-directed motility of lysosomes along microtubules. BORC comprises eight subunits and is linked via the myrlysin subunit to the lysosomal membrane. BORC may serve as a guanine nucleotide exchange factor for Arl8 and is required to recruit the small GTPase to lysosomes. Arl8-GTP is thought to bind the RUN domain of the adaptor SKIP that recruits KIF5 (kinesin-1) via kinesin light chain 2 (KLC2). (C) Schematic with lysosome distribution in cortical neurons from control mice (+Borcs7), Borcs7$^{-/-}$ animals (–Borcs7), and mice homozygous for the spontaneous Borcs7$^{Q87X/Q87X}$ mutation (Borcs-Q87X). Cartoons are based on work described in [2,5,6,9] and are limited to proteins and pathways relevant for the discussion of the Snouwaert paper.

the brain. Experiments with transgenic animals expressing one or both Borcs7-Q87X alleles and knockout animals revealed that Borcs7-Q87X retains some level of function since pups of the Borcs7$^{-/-}$ animals did not feed and died within 12 h after birth.

Snouwaert *et al*. then focused on lysosome distribution and dynamics in cortical neurons prepared from embryos of the various mouse strains. LAMP-1-containing lysosomes clustered primarily in the cell body and were absent in the axon of cells from Borcs7$^{-/-}$ while KIF5A had a more

diffuse localization, consistent with a requirement for BORC in KIF5A's association with lysosomes. Borcs$^{Q87X/Q87X}$ neurons had an intermediate phenotype (Figure 1C). In live cells, antegrade motility of lysosomes was most severely affected in the Borcs7 knockout and moderately in Borcs$^{Q87X/Q87X}$ neurons. Collectively these phenotypes are consistent with data from the Bonifacino laboratory in hippocampal neurons.

Why does a missense mutation in Borcs7 lead to a lysosome motility phenotype? In the absence of a suitable antibody, the authors could not address directly whether the truncated endogenous Borcs7-Q87X protein is actually expressed and whether the stoichiometry and expression levels of BORC and the BLOC-1 complex are affected. Transfected HA-Borcs7-Q87X, however, had a fourfold-shorter half-life, suggesting that reduced expression may be the underlying cause of the motor deficits in mice.

As so often, this paper also leaves us with new questions to be answered. Given the increased incidence of spheroid bodies containing organelles at various stages of degradation, it is imperative to study autophagic pathways in more detail in neurons of the Borcs$^{Q87X/Q87X}$ mice. Another question concerns the tissue specificity of the phenotype. Although expression of Borcs7 is higher in macrophages than in neurons, the Borcs$^{Q87X/Q87X}$ animals did not have an obvious lysosome positioning nor immunological phenotype. For that matter it would be interesting to challenge the immune system of these mice and then interrogate immune cell homeostasis, macrophage functions, and lysosome distribution.

Taken together, this study may contribute to the understanding of human disease especially because Borcs7 has been reported as a molecular risk factor in the 10q24.32 schizophrenia-associated locus [10].

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