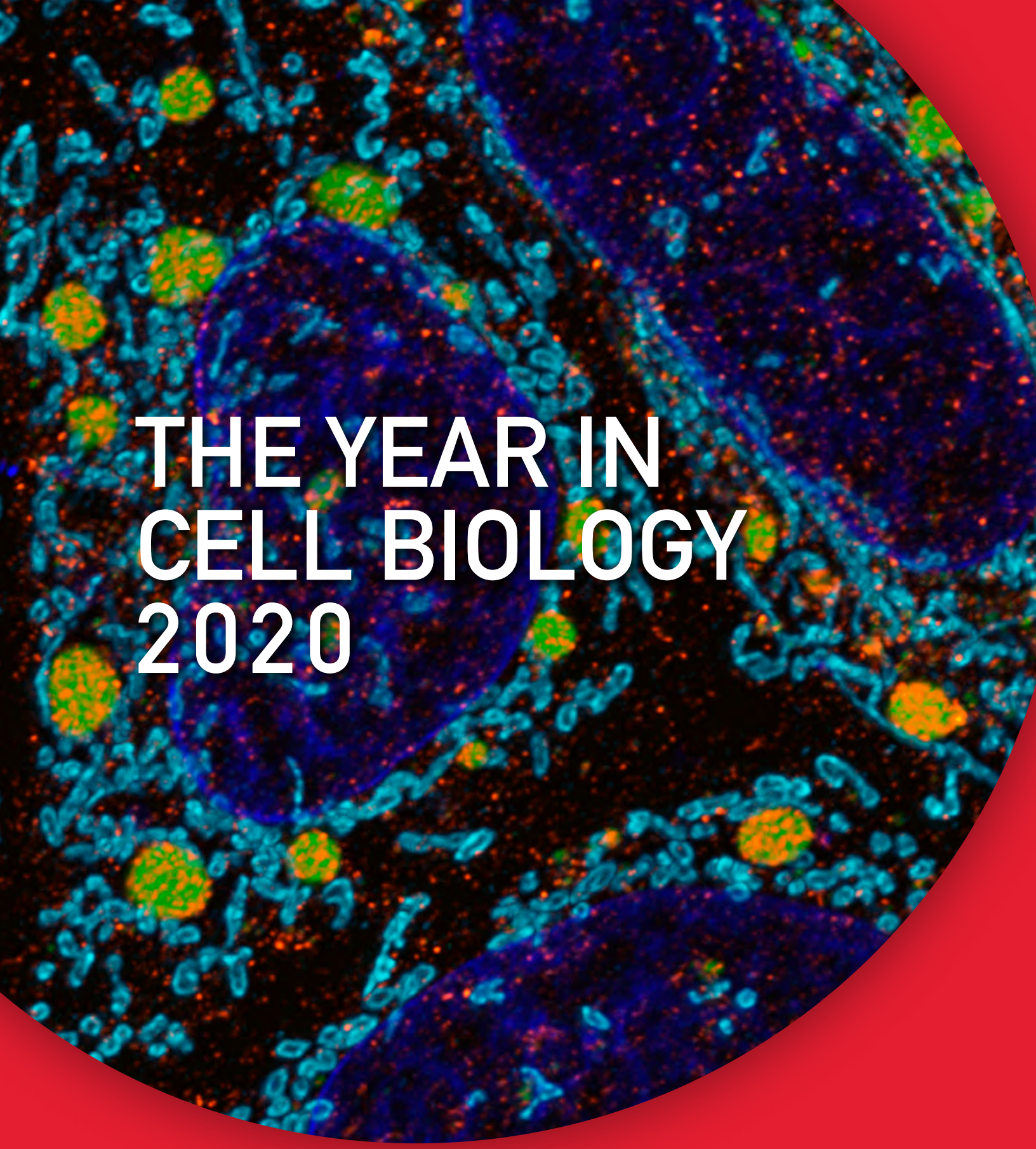
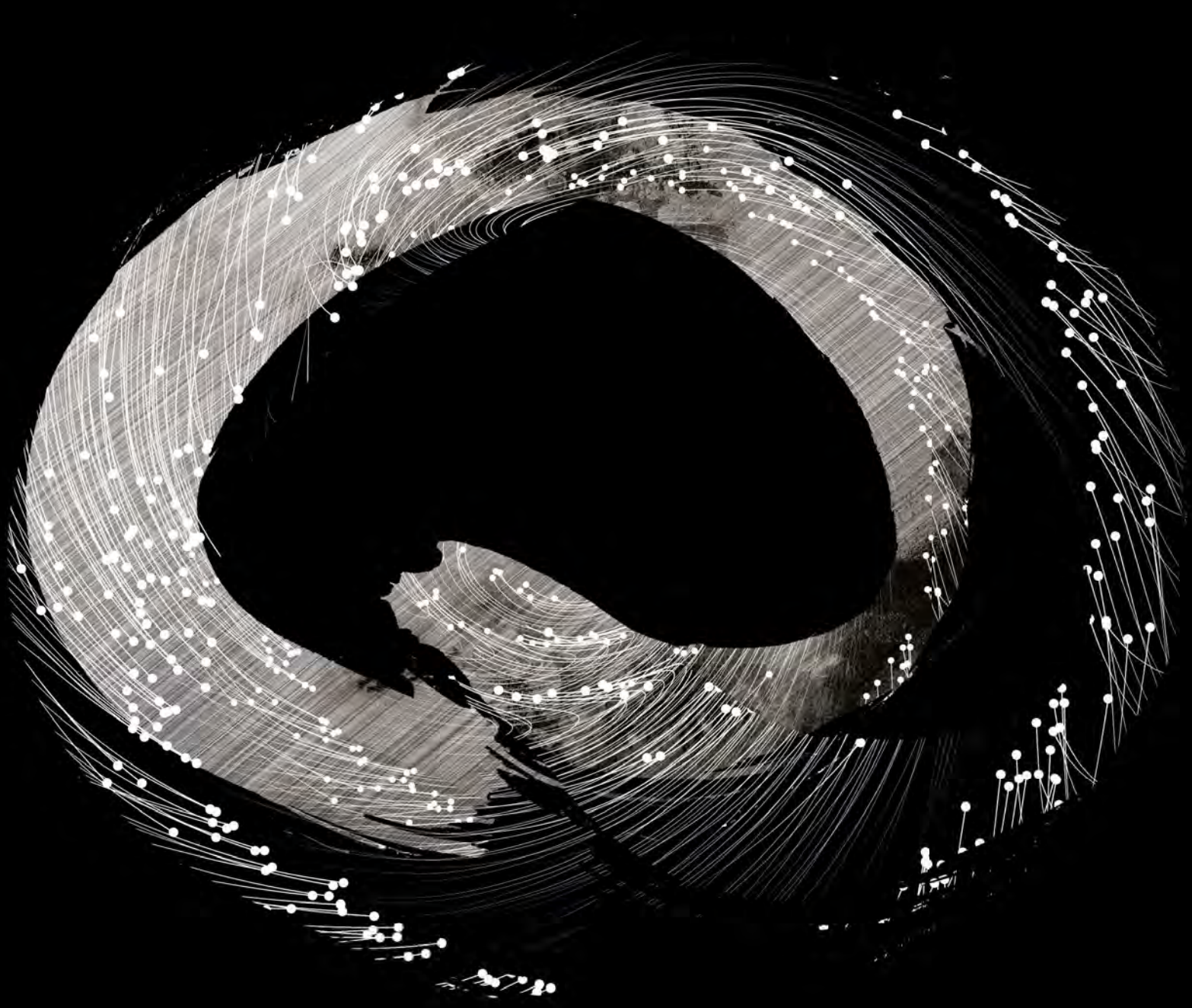


THE YEAR IN CELL BIOLOGY 2020

JCB Journal of Cell Biology



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JCB Journal of Cell Biology

THE YEAR IN CELL BIOLOGY 2020

We are pleased to present this collection of exceptional research published over the course of the last year in the *Journal of Cell Biology* (*JCB*). As always, our goal with this collection is to feature papers from across the breadth of cell biology and to highlight the articles that most captured the attention and interest of our readers. We therefore considered the number of times full-text articles were downloaded in the first three months after their initial publication when making our selection.

Although this collection cannot provide a full and comprehensive overview of all of the many exciting advances that have appeared in the pages of our journal, it does serve to offer a representative glimpse of the findings that our readers found most interesting this past year. From a study that demonstrates how cancer cells can "reprogram" natural killer cells to facilitate metastasis to work that reveals a role for optineurin and ATG9A in modulating ubiquitin-induced mitophagy, these articles showcase a fraction of the diversity of the *JCB* community. Furthermore, with articles that examine the mechanisms by which microtubules prevent cell rupture during amoeboid migration and the role of proteasome distribution in regulating the GABAergic response switch in neurons, this collection provides something for everyone in our diverse readership.

Needless to say, this year has been unlike any other in recent memory, and the COVID-19 pandemic has greatly affected every aspect of our lives, including the scientific community's ability to conduct research both safely and effectively. Despite this, the papers presented in this collection, as well as all of the work published over the past year, serve as a testament to the ingenuity, fortitude, and dedication of the cell biological community.

We are eternally grateful to the many people who make it possible for us to publish some of the very best cell biological studies in the world: our reviewers, who volunteer their time and energy throughout the year, to you, our readers, and most especially to our authors. It is truly our privilege to publish all of the papers you see in *JCB*. We thank you for continuing to submit your very best work to us.

We hope you enjoy reading this collection.

Brochure articles by Ben Short, PhD

Design by Yuko Tonohira

On the cover: A confocal maximum intensity projection image of HeLa cells. Core autophagy protein ATG9A vesicles (orange) are recruited to liquid droplets composed of Optineurin (green) and ubiquitin chain. Optineurin–ATG9A interaction is critical for the selective degradation of mitochondria (cyan). Nuclei are labeled in blue. Image See the associated article on page 12.

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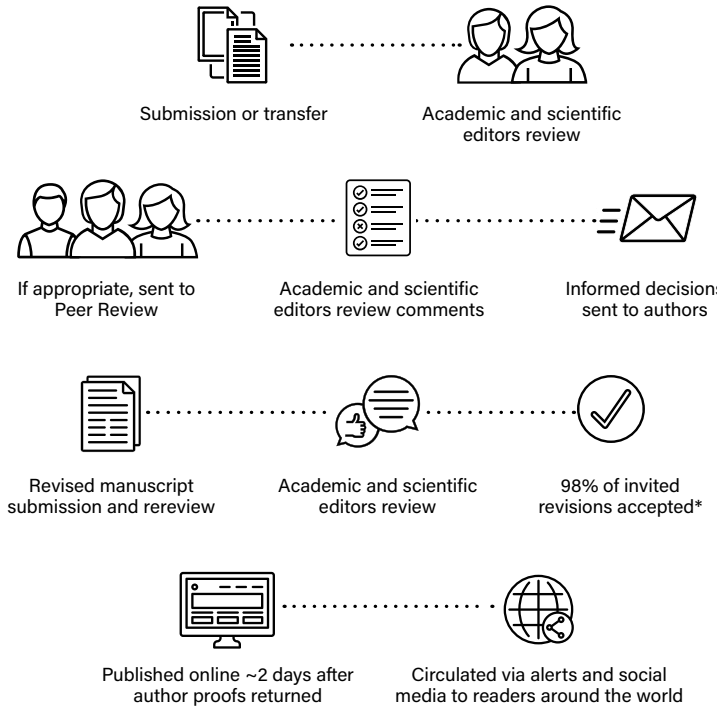

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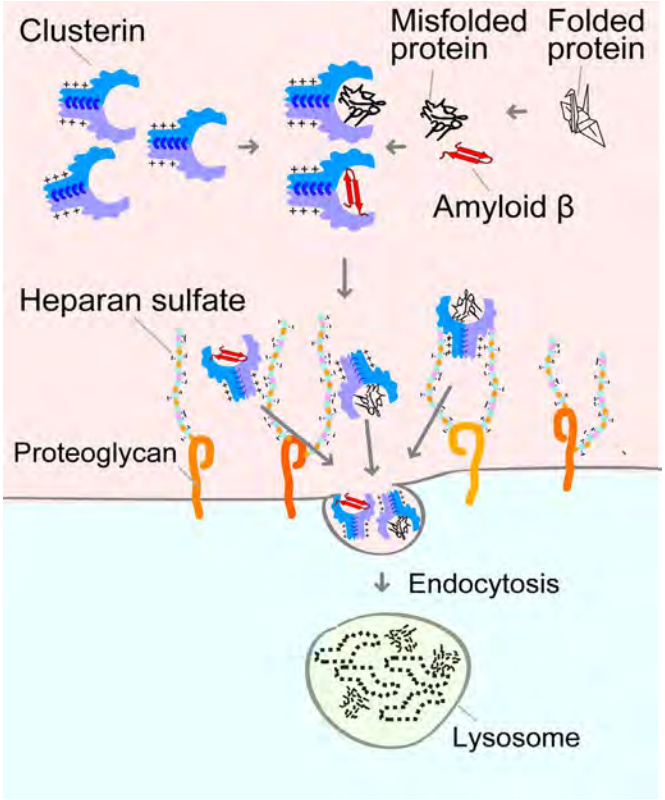
JCB Journal of Cell Biology

A SYSTEM TO REMOVE ABERRANT EXTRACELLULAR PROTEINS

The chaperone Clusterin works in combination with heparan sulfate proteoglycans to bring misfolded proteins into cells for degradation



A model of the CRED pathway in which Clusterin and heparan sulfate bring aberrant extracellular proteins, including amyloid β, into cells where they can be degraded by lysosomes. © 2020 Itakura et al.

A number of diseases are believed to be caused by the gradual buildup of misfolded proteins that can aggregate together and damage neurons and other cells in the body. To help prevent this damage, cells have developed numerous quality control systems that recognize misfolded proteins within the cell and either fold them back into their correct shape or degrade them before they start to aggregate.

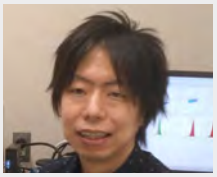
"However, approximately 11% of human proteins exist outside of the cell, where they are subjected to even more stresses that may cause them to misfold," says Eisuke Itakura, an assistant professor in the Department of Biology at Chiba University in Japan. "In addition, Alzheimer's disease, the most prevalent cause of dementia affecting 47.5 million people worldwide, is characterized by aggregates of amyloid β protein in the extracellular space. Despite this, how aberrant extracellular proteins are degraded remains poorly understood."

A chaperone protein called Clusterin can bind to misfolded extracellular proteins and prevent them from aggregating. Itakura and colleagues discovered that Clusterin can escort misfolded proteins into the cell and deliver them to the lysosomes for degradation. Using a genome-wide CRISPR-based screen, the researchers found that, after binding to misfolded proteins, Clusterin enters cells by interacting with heparan sulfate proteoglycans, a large class of proteins that are present on the surface of almost all human cells. This interaction requires a group of conserved, positively-charged residues on Clusterin that electrostatically interact with negatively-charged heparan sulfate.

Itakura and colleagues found that, together, Clusterin and heparan sulfate proteoglycans allow many different cell types to internalize and degrade a wide variety of misfolded extracellular proteins. "We therefore think that this pathway is a general extracellular protein quality control system responsible for the clearance of misfolded proteins from diverse tissues and body fluids," Itakura says. The researchers named this system the chaperone- and receptor-mediated extracellular protein degradation (CRED) pathway.

Intriguingly, Itakura and colleagues found that the CRED pathway can import amyloid β into cells for degradation. Mutations in the gene encoding Clusterin have been linked to an increased risk of developing Alzheimer's disease, and experiments in rats have shown that injecting Clusterin into the brain can prevent amyloid β–induced neurodegeneration. "Our results therefore suggest new avenues for the possible treatment or prevention of disorders such as Alzheimer's disease that are associated with aberrant extracellular proteins," Itakura says.

RESEARCHER DETAILS



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ORIGINAL PAPER

Itakura, E., M. Chiba, T. Murata, and A. Matsuura. 2020. Heparan sulfate is a clearance receptor for aberrant extracellular proteins. *J. Cell Biol.* 219: e201911126.

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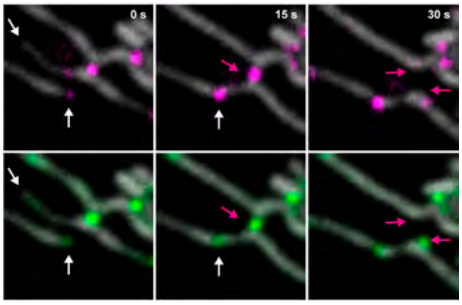
JCB Journal of Cell Biology

ER CONTACTS COORDINATE MITOCHONDRIAL DYNAMICS

Mitochondrial fission and fusion machineries colocalize at ER membrane contact sites, where they can rapidly modulate mitochondrial morphology in response to metabolic cues



Mfn1 (green) and Drp1 (magenta) colocalize at ER MCSs, where they mediate, respectively, the fusion (white arrows) and fission (magenta arrows) of mitochondria (grey).

The steady–state morphology of the mitochondrial network within cells is maintained by a balance of mitochondrial fission and fusion. Disrupting this balance causes the mitochondrial network to become either fragmented or elongated, potentially resulting in altered cell metabolism and disease.

Mitochondrial fission preferentially occurs at sites where the mitochondria contacts the ER. The dynamin-related GTPase Drp1 accumulates at these membrane contact sites (MCSs), and drives mitochondrial constriction and division. Mitochondrial fusion is also regulated by dynamin-related GTPases—the mitofusins Mfn1 and Mfn2 promote outer membrane fusion while Opa1 controls fusion of the inner mitochondrial membrane—but how these GTPase are positioned to define the site of fusion is unknown. However, fission and fusion may be spatially coordinated because mitochondria can sometimes undergo "transient" fusions, in which two mitochondria fuse and share their contents, before separating again to resume their original morphology.

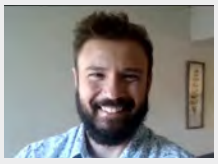
"We hypothesized that if fission and fusion are coordinated, then the mitochondrial fusion machinery might also assemble at ER MCSs," explains Gia Voeltz, a Howard Hughes Investigator and Professor at the University of Colorado, Boulder.

Using a variety of sophisticated imaging techniques, Voeltz and colleagues, including first author Robert Abrisch, found that mitofusins do, in fact, accumulate at ER MCSs and that these contact sites mark the location of ~90% of mitochondrial fusion events. "That's strikingly similar to the 88% of mitochondrial fission events scored to occur at ER MCSs," Voeltz says.

Further experiments revealed that Drp1 and Mfn1 colocalize at ER MCSs and that a fusion event at one site is often followed by fission at the exact same place. Voeltz and colleagues suspected that these hotspots of mitochondrial dynamics might be able to rapidly respond to metabolic cues and could, for example, allow damaged mitochondria to quickly recover their function by transiently fusing and exchanging components with neighboring, healthy mitochondria. Indeed, the researchers found that ER MCSs often mark the boundaries between polarized (healthy) and depolarized (unhealthy) segments of mitochondria, and that depolarized mitochondria can restore their membrane potential by fusing with healthy neighbors at ER MCSs.

"Depolarization of individual mitochondria pushes the reaction at ER MCSs toward fusion, resulting in the recovery of membrane potential," Voeltz says. "But other signals may push the reaction toward fission. "Future studies will investigate whether fission and fusion rates are determined by the relative recruitment and/or posttranslational modifications of the different machineries at ER MCSs, by small signaling molecules like $Ca2^{+}$, or by the recruitment of activators or inhibitors of these machineries."

RESEARCHER DETAILS



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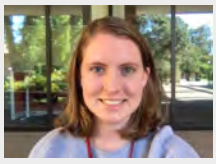

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ORIGINAL PAPER

Abrisch, R.G., S.C. Gumbin, B.T. Wisniewski, L.L. Lackner, and G.K. Voeltz. 2020. Fission and fusion machineries converge at ER contact sites to regulate mitochondrial morphology. *J. Cell Biol.* 219: e201911122.

https://doi.org/10.1083/jcb.201911122

JCB Journal of Cell Biology

VPS13 FORMS A LIPID CHANNEL BETWEEN MEMBRANES

Cryo-EM of VPS13 N-terminal fragment reveals a hydrophobic groove that may allow bulk lipid flow between organelles

Lipids constantly need to be exchanged between the various different membranes of eukaryotic cells. For example, most membrane lipids are synthesized in the ER and must then be delivered to other organelles. This is carried out, in part, by lipid transfer proteins that localize to the membrane contact sites between organelles and act as shuttles, extracting lipid molecules from one organelle and then depositing it in the membrane of the other.

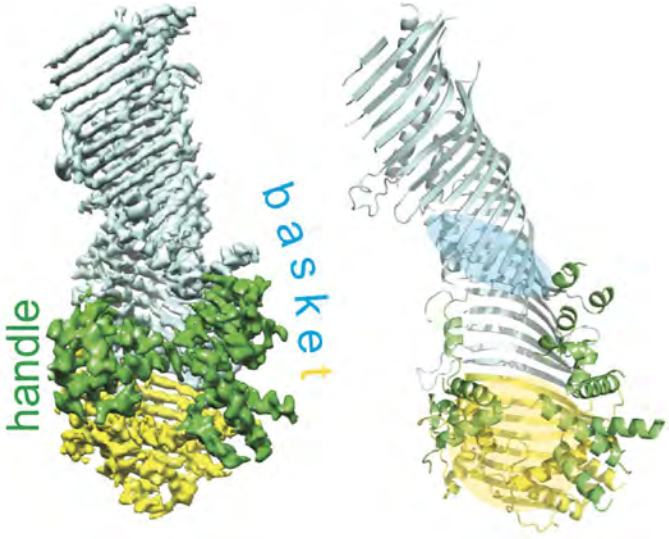
"Typically, these lipid transport proteins comprise domains resembling lidded teacups, each with a hydrophobic cavity that accommodates one or two lipid molecules," explains Karin Reinisch, a professor at Yale School of Medicine. This limited capacity is unlikely to be sufficient when large amounts of lipid need to be transferred rapidly, such as during the assembly of the autophagosome during autophagy, or the formation of the prospore membrane during yeast meiosis.

The VPS13 family of lipid transport proteins may work differently, however. These very large proteins are thought to tether organelles together at membrane contact sites and can bind tens of lipid molecules at a time. Budding yeast Vps13p is required for prospore membrane formation, and, in humans, there are four family members, some of which are linked to severe neurodegenerative disease.

Reinisch and colleagues, including first author PeiQi Li, carried out a single-particle cryo-EM reconstruction of a ~160-kD N-terminal fragment of the VPS13 protein of the filamentous fungus *Chaetomium thermophilum*. The researchers found that the fragment adopts a basket-like structure whose interior is lined with hydrophobic residues. Thus, the N-terminal region of VPS13 forms an extended channel, ~160 Å in length, that would be well-suited to solubilizing multiple lipid molecules at a time.

Instead of shuttling lipids between membranes, VPS13 proteins might therefore act like bridges, tethering organelles together via their N- and C-termini and allowing lipids to flow from one membrane to the other through their hydrophobic channels. As an initial test of this idea, Li et al. created mutant versions of budding yeast Vps13p in which sections of the channel were switched to hydrophilic residues instead of hydrophobic ones. These *vps13* mutants could still bind lipids but they were unable to support sporulation, indicating that they are incapable of transferring lipids during prospore formation.

"The size of the VPS13 lipid-binding cavity and, hence, the ability of these proteins to accommodate many lipids simultaneously suggests a role in bulk lipid transfer," says Reinisch, who notes that the VPS13-like protein ATG2 may act similarly to promote autophagosome assembly during autophagy.



The density map (left) and secondary structure (right) of $VPS_{131-1390}$ show the fragment's basket-like structure and the extended, hydrophobic channel that may facilitate lipid flow between membranes. © 2020 Li et al.

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ORIGINAL PAPER

Li, P., J.A. Lees, C.P. Lusk, and K.M. Reinisch. 2020. Cryo-EM reconstruction of a VPS13 fragment reveals a long groove to channel lipids between membranes. *J. Cell Biol.* 219: e202001161.

https://doi.org/10.1083/jcb.202001161

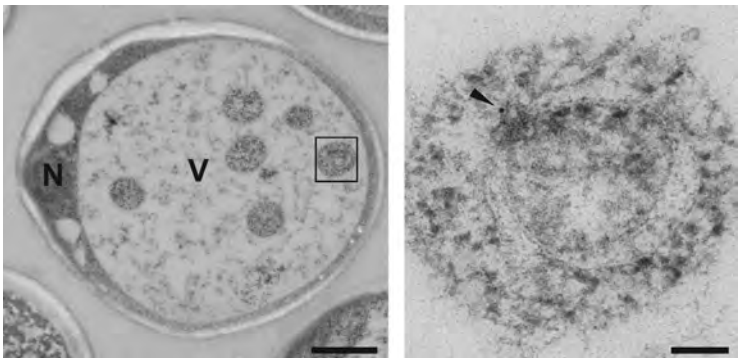
JCB Journal of Cell Biology

AUTOPHAGY TARGETS NUCLEAR PORE COMPLEXES

Several different pathways selectively target NPCs and nucleoporins for autophagic degradation upon inactivation of the TORC1 kinase complex

Composed of ~30 different nucleoporin proteins, nuclear pore complexes (NPCs) span the inner and outer membranes of the nuclear envelope and mediate transport between the nucleus and cytoplasm, thereby playing critical roles in gene expression, cell growth and division. Defects in NPC function have been linked to aging and a variety of illnesses including Alzheimer's disease and cancer. Cells are therefore likely to employ a variety of quality control mechanisms to ensure they maintain an appropriate number of functional NPCs. "However, the cellular systems that degrade the NPC or nucleoporins are still largely unknown," says Hitoshi Nakatogawa from the Tokyo Institute of Technology.

Nakatogawa and colleagues, including first author Yui Tomioka, investigated whether NPCs are broken down by autophagy, a process in which cellular components are engulfed by autophagosomes and delivered to lysosomes for degradation. The researchers found that inducing the autophagy pathway in budding yeast by inactivating the TORC1 kinase complex resulted in the degradation of multiple nucleoporins. Blocking degradation caused these NPC components to accumulate in vacuoles—the yeast equivalent of lysosomes—where they appeared to be embedded within nuclear envelope–derived double-membrane vesicles. This suggests that nucleoporins can be engulfed by autophagosomes and delivered to lysosomes while they are still assembled into NPCs.



In yeast cells lacking vacuolar proteases, TORC1 inactivation leads to the accumulation of Nup159-containing structures (arrowhead) embedded in nuclear envelope-derived double-membrane vesicles located inside autophagosomes that have been delivered to the vacuole. © 2020 Tomioka et al.

Though autophagy can be a nonselective process, many cellular components are targeted to autophagosomes by specific autophagy receptors that interact with either Atg11, which then initiates autophagosome formation, or Atg8, a protein located on forming autophagosomal membranes. Tomioka et al. found that yeast lacking Atg11, or yeast expressing an Atg8 mutant unable to bind autophagy receptors, failed to degrade nucleoporins upon TORC1 inactivation, indicating that NPCs/nucleoporins are selectively targeted to autophagosomes.

Nakatogawa and colleagues determined that some NPCs are targeted by a previously identified pathway called nucleophagy, in which nuclear envelope–derived vesicles are recruited to autophagosomes via an autophagy receptor in the outer nuclear membrane called Atg39. But NPCs can also be targeted via a different pathway, that the researchers named NPC-phagy, involving an as yet unknown autophagy receptor.

In addition, Tomioka et al. found that one nucleoporin, Nup159, can bind directly to Atg8. Disrupting this interaction impaired the turnover of Nup159 but had no effect on the degradation of other nucleoporins, suggesting that nucleoporins not assembled into NPCs can also be targeted to autophagosomes via a process the researchers dubbed nucleoporinophagy.

Together, these different selective autophagy pathways are likely to help cells maintain the structural and functional integrity of their NPCs. "Our study provides a foundation for understanding the molecular mechanisms and physiological/pathological significance of these autophagy pathways in various organisms," Nakatogawa says.

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ORIGINAL PAPER

Tomioka, T., T. Kotani, H. Kirisako, Y. Oikawa, Y. Kimura, H. Hirano, Y. Ohsumi, and H. Nakatogawa. 2020. TORC1 inactivation stimulates autophagy of nucleoporin and nuclear pore complexes. *J. Cell Biol.* 219: e201910063.

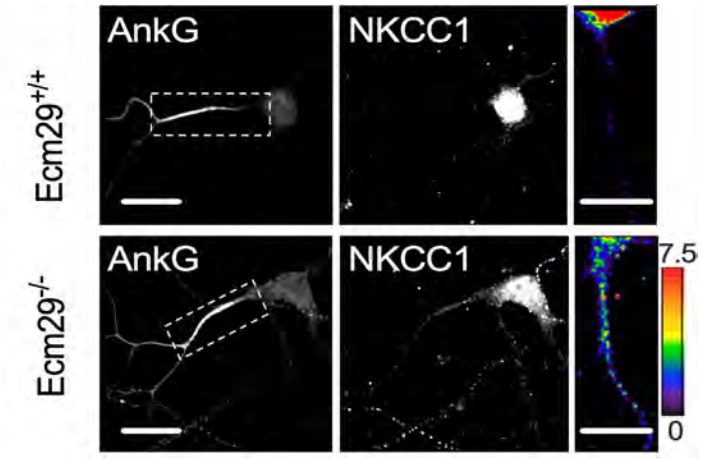
https://doi.org/10.1083/jcb.201910063

PROTEASOME DISTRIBUTION MODULATES GABA RESPONSES IN DEVELOPING NEURONS

The adaptor protein Ecm29 positions proteasomes at the axon initial segment to promote degradation of the chloride transporter NKCC1 and limit neuronal excitability

The response of mouse neurons to the neurotransmitter GABA changes from excitatory to inhibitory during the first few weeks after birth, a developmental switch that helps to establish the lifelong activity of local neuronal circuits. The change in GABA response is due to a reduction in the intracellular chloride concentration in neurons that is thought to be driven by increased expression of the chloride exporter KCC2 and decreased levels of the chloride importer NKCC1. "However, how NKCC1 levels are down-regulated during the excitatory-to-inhibitory GABA transition remains unclear," says Pei-Lin Cheng, an Associate Research Fellow at the Institute of Molecular Biology in Taiwan.

Cheng and colleagues, including first author Min Lee, discovered that developing neurons regulate NKCC1 levels and the GABA response by localizing the protein-degrading proteasome to the axon initial segment (AIS), a specialized membrane domain that forms around the same time as the GABAergic switch. Composed of various scaffold proteins such as ankyrin G, the AIS segregates the axonal and somatodendritic compartments of neurons and controls action potential firing. Cheng and colleagues found that, as the AIS assembles in developing hippocampal and cortical neurons, an adaptor protein called Ecm29 tethers proteasomes to the AIS by binding to ankyrin G.



NKCC1 levels in the AIS region of neurons are increased in the absence of the proteasome adaptor Ecm29 (bottom). A linear pseudocolored scale shows the intensity of NKCC1 staining within the AIS (marked by ankyrin G). © 2020 Lee et al.

Neurons lacking Ecm29 showed reduced protein turnover at the AIS, and Ecm29-deficient mouse brains showed higher levels of NKCC1 that declined more slowly during the postnatal period. NKCC1 levels were particularly elevated in the AIS region of neurons. As a result, the switch from excitatory to inhibitory GABA responses was delayed in Ecm29-knockout mice, causing the animals' neurons to be hyperexcitable during this critical period in neuronal development.

"We hypothesized that prolonged NKCC1 expression/excitatory GABA responsiveness in Ecm29-deficient immature neurons puts mice at higher risk of seizures," Cheng says. Indeed, Lee et al. found that Ecm29-null mice were more susceptible to chemical-induced seizures, but were protected if treated with a potent NKCC1 inhibitor during the first few weeks of life.

"Our findings provide a developmental mechanism linking timing of AIS formation, proteasome transport, and subcellular proteostasis to the critical window of the GABAergic switch, which governs excitability of maturing hippocampal/cortical neurons and is mediated by Ecm29," says Cheng, who notes that changes in AIS-associated proteins have been linked to various neurodevelopmental and psychiatric disorders, including epilepsy.

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ORIGINAL PAPER

Lee, M., Y.-C. Liu, C. Chen, C.-H. Lu, S.-T. Lu, T.-N. Huang, M.-T. Hsu, Y.-P. Hsueh, and P.-L. Cheng. 2020. Ecm29-mediated proteasomal distribution modulates excitatory GABA responses in the developing brain. *J. Cell Biol.* 219: e201903033.

https://doi.org/10.1083/jcb.201903033

JCB Journal of Cell Biology

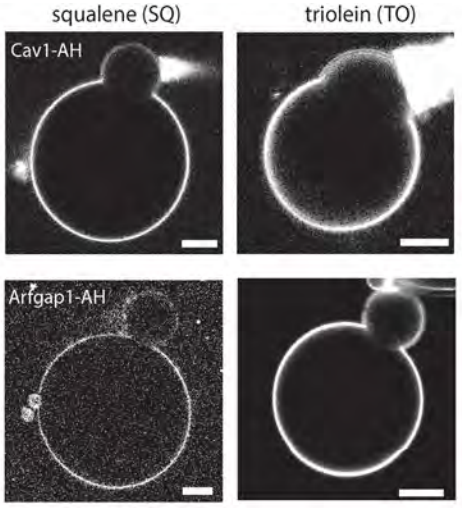
NEUTRAL LIPIDS CONTROL PROTEIN RECRUITMENT TO LIPID DROPLETS

The neutral lipid core content is determinant to the affinity of amphipathic helices for artificial lipid droplets

Lipid droplets (LDs) store neutral lipids and control their hydrolysis to meet the cell's demands for energy or membrane synthesis. Formed at the ER—the major site of lipid synthesis in eukaryotic cells—LDs are composed of a central neutral lipid core, surrounded by a phospholipid monolayer containing numerous regulatory proteins.

Many of these proteins bind to LDs via amphipathic helices (AHs), but how these helices selectively target LDs—or even specific subsets of LDs—remains unclear. After all, AHs can also target proteins to the cell's many different membranes by inserting themselves into the hydrophobic core of lipid bilayers. "In the case of LDs, AHs have to expose their hydrophobic moieties to the neutral lipid core. Thus, interactions between the hydrophobic amino acids of an AH with neutral lipids could be a major driving force for LD targeting," says Abdou Rachid Thiam, a professor at the École Normale Supérieure in Paris.

To test this idea, Thiam and graduate student Aymeric Chorlay measured the binding of various AHs to artificial LDs embedded within the bilayer of giant vesicles in vitro (mimicking the emergence of cellular LDs from within the ER bilayer). Chorlay and Thiam found that AH peptides derived from LD proteins differentially bound to artificial LDs, depending on the neutral lipid making up the droplet's core.



The AHs of the LD proteins Cav1 and Arfgap1 are recruited to artificial LDs budding from the bilayer of giant vesicles in vitro, preferentially binding LDs composed of the neutral lipid triolein, compared with LDs made from squalene. © 2020 Chorlay and Thiam

"We found that neutral lipid composition determines the binding level of AHs, and the ability of AHs to discriminate between neutral lipids relies on their amphipathic amino acid sequence," Thiam explains. For example, mutations in the AH of the LD protein Cav1 either enhanced or abolished its preference for artificial LDs composed of the neutral lipid triolein.

Chorlay and Thiam also determined that the phospholipids surrounding LDs influence AH binding by regulating the access of AHs to the neutral lipid core. However, while reducing the density of phospholipids on artificial LDs increased AH binding, it didn't change the AHs's relative preference for LDs composed of particular neutral lipids.

"Overall, our data highlight an undervalued contribution of neutral lipids in controlling the binding of AHs to the surface of LDs," Thiam says. "The phospholipid packing density simply regulates the amount of exposed neutral lipids, which dictates the binding level of AHs. Clearly, the full picture of how AHs selectively bind to LDs in a cellular context is not completely resolved yet, but our data bring us a major step closer to it."

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ORIGINAL PAPER

Chorlay, A. and A.R. Thiam. 2020. Neutral lipids regulate amphipathic helix affinity for model lipid droplets. *J. Cell Biol.* 219: e201907099.

https://doi.org/10.1083/jcb.201907099

JCB Journal of Cell Biology

UNRAVELLING THE CRITICAL STEPS IN MITOPHAGY

Study reveals that mitochondrial ubiquitination and an interaction between OPTN and ATG9A are crucial for inducing the selective degradation of damaged mitochondria

Damaged mitochondria are quickly eliminated by a process called mitophagy, a selective type of autophagy in which the organelles are engulfed by autophagosomes and delivered to lysosomes for degradation. Two proteins linked to familial Parkinson's disease—the ubiquitin kinase PINK1 and the ubiquitin ligase Parkin—recognize depolarized mitochondria and rapidly coat the outer membrane with ubiquitin chains that act as a signal for the assembly of autophagosomal membranes around the damaged organelles.

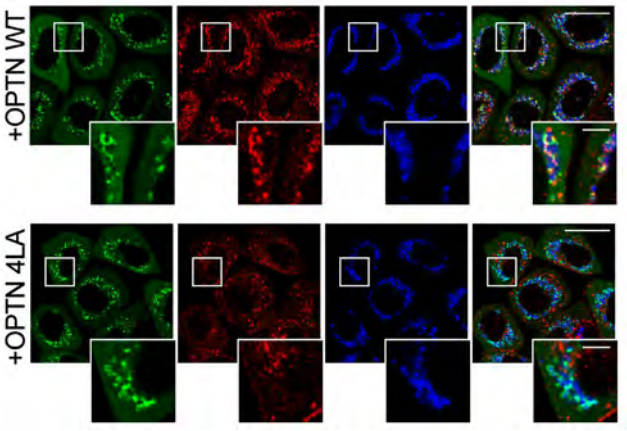
"However, it was unclear whether Parkin and PINK1 function solely in the ubiquitination of dysfunctional mitochondria, or if they also physically communicate with the core autophagy machinery," says Koji Yamano from the Tokyo Metropolitan Institute of Medical Science.

To address this question, Yamano and colleagues, including co-senior author Noriyuki Matsuda, devised ways to tag mitochondria with ubiquitin chains independently of PINK1, Parkin, or mitochondrial depolarization. The researchers found that the addition of short, linear ubiquitin chains to mitochondria was sufficient to induce mitophagy and mitochondrial degradation. "This demonstrates that the Parkin–PINK1 system is essential for ubiquitination of damaged mitochondria but is not required for autophagy activation per se," Yamano says.

Ubiquitinated mitochondria are thought to be linked to autophagosomes by five different autophagy adaptors that, in addition to binding ubiquitin, can also bind to ATG8 proteins on the autophagosomal membrane. Two of these adaptors—OPTN and NDP52—appear to be particularly crucial for mitophagy. Surprisingly, however, Yamano and colleagues determined that this isn't because OPTN and NDP52 have a higher affinity for ATG8 proteins.

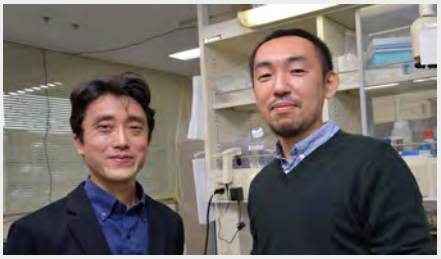
Instead, the researchers discovered, OPTN plays a critical role in mitophagy by recruiting ATG9A vesicles to ubiquitinated mitochondria (these vesicles are thought to supply components that aid autophagosome assembly). Yamano et al. found that the leucine zipper domain of OPTN binds to ATG9A. Mutations in this domain reduced the ability of OPTN to support mitophagy even more than mutations in OPTN's ATG8-binding domain.

Other recent studies have shown that the other crucial mitophagy adaptor, NDP52, can recruit the autophagy-activating ULK complex to damaged mitochondria. "Our study, in conjugation with these previous reports, indicates that the association of OPTN (and NDP52) with ubiquitinated mitochondria promotes the formation of an initial platform that triggers the assembly of different autophagy core units through multivalent interactions," Yamano says. "These functionalities thus fulfill a critical role for de novo autophagosomal membrane formation close to the ubiquitinated cargo."



Following mitochondrial depolarization, ATG9A (red) is recruited to mitochondria (blue) in cells expressing wild-type OPTN (green, top row) but not in cells expressing OPTN with a mutated leucine zipper domain (green, bottom row). © 2020 Yamano et al.

RESEARCHER DETAILS



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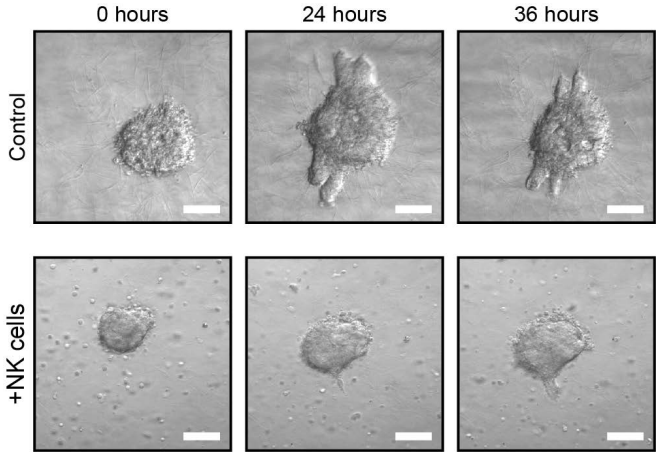
JCB Journal of Cell Biology

BREAST CANCER CELLS TURN KILLER IMMUNE CELLS INTO ALLIES

Natural killer cells are reprogrammed by breast cancer cells to promote metastasis



Initially, the presence of NK cells (bottom row) restricts the growth of tumor organoids in 3D collagen matrices. But after 36 h, the cancer cells reprogram the NK cells, and the tumor starts to invade its surroundings. © 2020 Chan et al.

Breast cancer cells spread to other parts of the body by invading the surrounding, healthy breast tissue until they reach the circulation, which can carry them to other tissues where they can form new metastatic tumors, a major cause of death in breast cancer patients. Natural killer (NK) cells are key components of the innate immune system that can recognize and kill cancer cells as they attempt to spread through the body.

"Breast cancer cells must overcome NK cell surveillance in order to form distant metastases," says Andrew Ewald, a professor of cell biology at Johns Hopkins University School of Medicine and co-director of the Cancer Invasion and Metastasis Program in the Sidney Kimmel Comprehensive Cancer Center. "However, we do not fully understand how breast cancer cells escape NK cell–mediated immunosurveillance during their transit through the circulation and the initial seeding of distant organs."

Ewald and colleagues, including first author Isaac Chan, discovered that, although metastasizing breast cancer cells are initially vulnerable to NK cells, they are quickly able to alter the behavior of their would-be killers, reprogramming them so that they actually promote the later stages of metastasis. Using several new assays to model metastasis in the laboratory as well as experiments in mice, the researchers found that, after they encounter tumor cells, human and mouse NK cells lose the ability to restrict tumor invasion and instead help cancer cells form new tumors.

NK cells exposed to tumors undergo dramatic changes, turning thousands of genes on and off and expressing different receptor proteins on their surface. Ewald and colleagues found that antibodies targeting two key receptor proteins on the surface of NK cells, called TIGIT and KLRG1, prevented NK cells from helping breast cancer cells seed new tumors. The FDA-approved drugs decitabine and azacitidine had similar effects, likely because they prevent large-scale changes in gene activity by inhibiting enzymes known as DNA methyltransferases.

The researchers found that combining decitabine or azacitidine treatment with anti-TIGIT or anti-KLRG1 antibodies was particularly effective at preventing NK cells from enhancing the metastatic potential of breast cancer cells.

"The synergistic effects of DNA methyltransferase inhibitors with receptor-blocking antibodies suggests a viable clinical strategy to reactivate tumor-exposed NK cells to target and eliminate breast cancer metastases," says Chan.

Ewald notes, "Combined with our observation that NK cells are abundant early responders to disseminated breast cancer cells, our data provide a preclinical rationale for the concept of NK cell–directed immunotherapies in the adjuvant setting for breast cancer patients with high risk of metastatic recurrence."

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ISG15 ACCELERATES REPLICATION FORK PROGRESSION

The ubiquitin-like molecule ISG15, which is induced by interferons and is often upregulated in cancer cells, can increase genome instability and sensitize cells to genotoxic drugs

ISG15 is strongly induced by type I and type III interferons in response to bacterial or viral infection. Though its amino acid sequence is very different, ISG15's 3D structure is similar to ubiquitin and, like ubiquitin, it can be conjugated to other proteins by E3 ligases. But increasing evidence suggests that ISG15 can modulate the host immune response by non-covalently binding to other proteins or even by acting as a cytokine secreted from cells.

ISG15 is also induced by DNA damage and is frequently overexpressed in cancer cells. "Elevated *ISG15* levels occur in many types of cancer and, in some cases, the robust expression of *ISG15* has been reported to support tumor growth," explains Lorenza Penengo from the Institute of Molecular Cancer Research at the University of Zurich. "However, its role in tumorigenesis is still controversial, and its mechanism of action is far from being clarified."

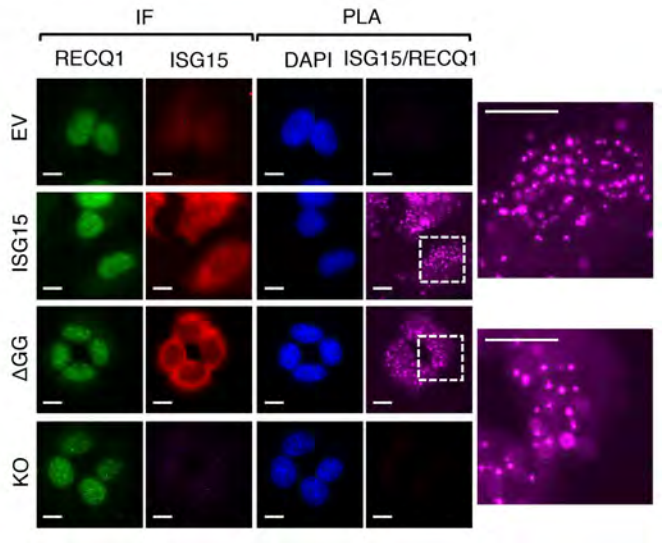
Penengo and colleagues, including first author Chiara Raso, discovered that ISG15 localizes to DNA replication forks, suggesting that it might modulate DNA replication. Inducing *ISG15* expression accelerated fork progression, whereas deleting the gene reduced the speed of DNA replication in several cancer cell lines that usually overexpress *ISG15*.

Raso et al. determined that ISG15 can regulate DNA replication fork progression through non-covalent mechanisms: overexpression of a mutant version incapable of being conjugated to other proteins still accelerated fork progression.

The researchers found that ISG15 associates with several proteins at replication forks, including a DNA helicase, RECQ1, that helps to restart stalled forks. "Depletion of RECQ1 completely abolished the accelerated replication fork progression induced by high levels of ISG15, suggesting that ISG15 may regulate RECQ1 function by unleashing its restart activity," Penengo says. Indeed, increased ISG15 levels promoted fork restart in a RECQ1-dependent manner.

Elevated *ISG15* might therefore be detrimental to cancer cells by causing DNA replication to continue in the presence of genotoxic drugs that would normally slow replication fork progression, resulting in genomic instability. Raso et al. found that cancer cells with high ISG15 levels were more sensitive to low doses of the chemotherapeutic agents camptothecin and cisplatin, because their replication forks continued unabated, leading to chromosome breakages and cell death.

"The increased activity of RECQ1 induced by high ISG15 levels may thus represent an important vulnerability that can be exploited for genotoxic anticancer treatments," Penengo says. "Furthermore, the evaluation of ISG15 levels in tumor samples may represent a predictive parameter to stratify patients in personalized cancer therapy."



Proximity ligation assays (magenta) show that ISG15 (red) associates with the DNA helicase RECQ1 (green) in cells, even when the cells express a mutant version of ISG15 (ΔGG) that cannot be covalently conjugated to other proteins.

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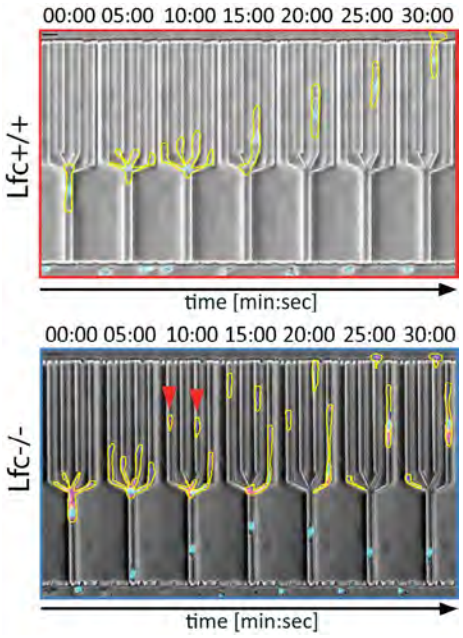
MICROTUBULES HELP MIGRATING CELLS KEEP THEIR SHAPE

Dendritic cells coordinate their movement through complex microenvironments by using the local depolymerization of microtubules to trigger the actomyosin-mediated retraction of protrusions

Cells navigate through complex tissues by sending out multiple protrusions to explore different paths, but protrusions extending in the "wrong" direction must then be retracted so that the cell can move forward without becoming entangled with its surroundings. Migrating fibroblasts are thought to coordinate their protrusions using mechanical signals transmitted via actomyosin-based stress fibers associated with focal adhesions, whereas neutrophils and other small leukocytes may use plasma membrane tension to relay signals from one protrusion to another.

How dendritic cells (DCs) control their shape as they surveil tissues for foreign antigens and deliver them to lymph nodes is unclear, however. DCs use an amoeboid mode of migration that is largely independent of adhesions and stress fibers, and they are too large and ramified for membrane tension to coordinate their protrusions. "We recently found that when DCs migrate through complex geometries, their nucleus acts as a mechanical gauge to lead them along the path of least resistance and the microtubule organizing center (MTOC), which is closely associated with the nucleus, is critical for this navigational task," says Michael Sixt from the Institute of Science and Technology in Austria.

Sixt and colleagues, including co-corresponding author Eva Kiermaier and first author Aglaja Kopf, therefore wondered whether microtubules nucleated from the MTOC might control the shape of migrating DCs. The researchers found that, as the nucleus and associated MTOC move into one protrusion and guide the cell forward, protrusions pointing in other directions lose their microtubules and retract. "Upon passage of the MTOC, sheer geometry may determine that all but the leading protrusion are cut off from microtubule supply because microtubules are too inflexible to find their way into curved, narrow, and ramified spaces," Kopf says.



A wild-type DC (top) successfully navigates through a branched microfluidic channel after sending out multiple protrusions to explore the various pathways. A DC lacking Lfc (bottom) fails to properly retract its exploratory protrusions and undergoes fragmentation (red arrowheads). © 2020 Kopf et al.

Kopf et al. determined that the local depolymerization of microtubules triggers the retraction of protrusions. The Rho GTPase exchange factor GEF-H1/Lfc associates with microtubules and is released when they depolymerize, leading to the activation of actomyosin contractility and protrusion retraction. Depleting Lfc impaired DC migration, often causing the cells to fragment as they failed to retract their protrusions and became entangled in their surroundings.

"We propose that microtubules serve as an internal explorative system of the cell that informs actomyosin whenever a peripheral protrusion locates too distant from the centroid and thereby initiates its retraction," Kiermaier says.

"We also found that DCs lacking both Lfc and microtubules have even more severe cell shape defects than cells lacking Lfc only, indicating that microtubule depolymerization induces cell retraction via additional modes that remain to be identified," Sixt adds.

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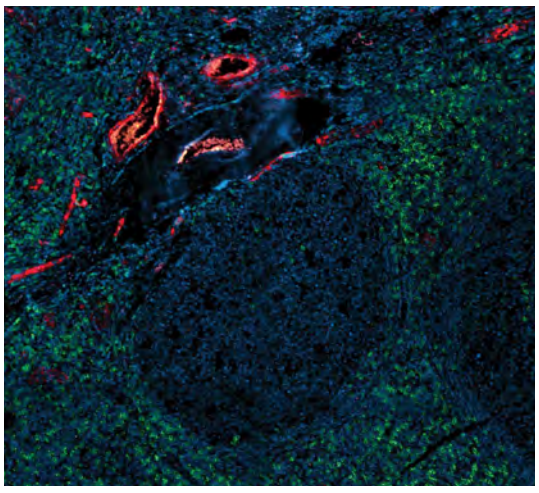

Human paraffin-embedded tonsil tissue co-stained with anti-human PNAd (clone MECA-79) Biotin, and anti-human CD8a (clone C8/144B) Alexa Fluor® 647 (green) followed by Streptavidin Spark YG™ 570 (red). Nuclei were counterstained with DAPI (blue).

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