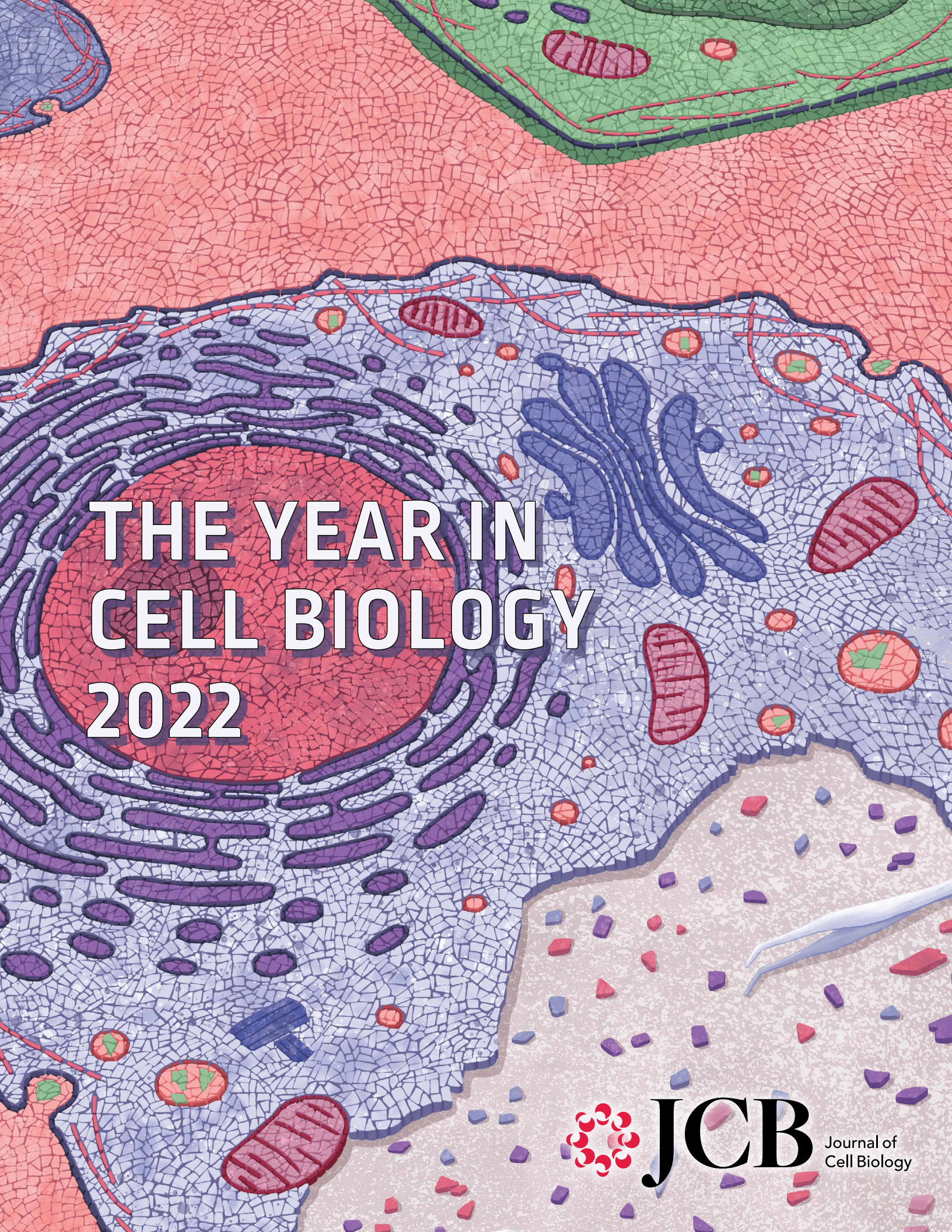
THE YEAR IN
CELL BIOLOGY
2022
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# THE YEAR IN CELL BIOLOGY 2022

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**Brochure articles by** Ben Short, PhD and Christina Szalinski, PhD

**Design by** Yuko Tonohira

**Cover artwork by** Joana C. Carvalho

We are pleased to offer a collection of outstanding articles published in the past year in the *Journal of Cell Biology.*

While no collection can capture the full breadth of the exciting research advances published over the last 12 months, the following articles showcase the exceptional diversity and quality of work that most interested our readers. From liquid crystalline phasing in lipid droplets to a survey of mammalian axons by cryo-electron microscopy, this collection contains transformative research that exemplifies the strength and impact of the *JCB* community.

This year, as research institutions fully reopen laboratories and conferences slowly return to in-person participation, the pages of *JCB* were filled with work that was completed under extraordinary challenges of institutional lockdowns. This collection honors the enormous challenges overcome by our authors since the spring of 2020 to publish their highest quality research. Indeed, this collection includes papers on the intracellular events that deliver mRNA from vaccines for translation inside cells as well as the ability of SARS-CoV-2 to interfere with cilia.

Every paper in *JCB* reflects the time and dedication of our reviewers, for which we are always grateful. We are also grateful to our many readers and, most especially, to the authors who continue to send us their best work. Your efforts make the journal possible. Every issue we publish represents work that the *JCB* community should be proud of, and we are truly honored to serve this wonderfully diverse and ever-growing community of scholars.

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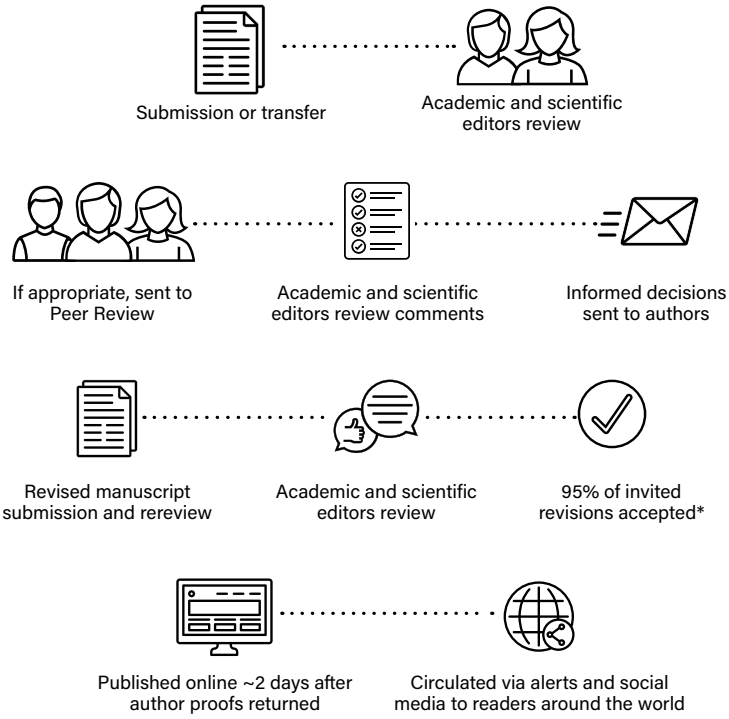


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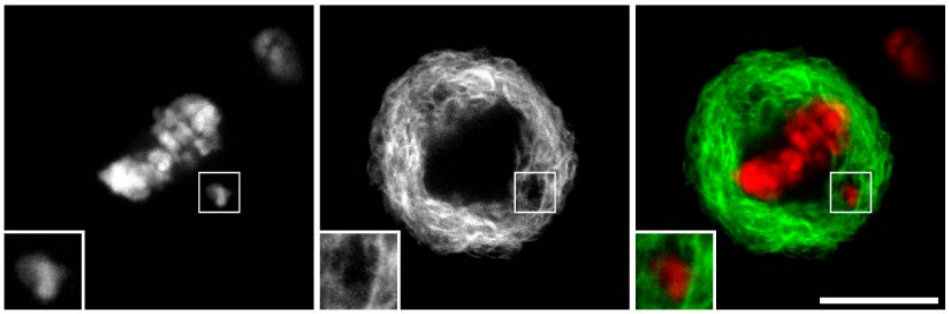
# ENDOMEMBRANES PROMOTE CHROMOSOME MISSEGREGATION

***Misaligned chromosomes can become ensheathed by intracellular membranes during mitosis, leading to missegregation and micronucleus formation.***

In order to segregate correctly during mitosis, chromosomes must attach to the mitotic spindle and align themselves at the metaphase plate. Errors or delays in this process can lead to cells with micronuclei and/or abnormal numbers of chromosomes, features that are frequently seen in cancer cells and are thought to contribute to tumor progression.



During mitosis, chromosomes (red) that lie outside of the central exclusion zone become ensheathed by endomembranes (green), impairing their alignment at the metaphase plate. 

At the onset of mitosis, the nuclear envelope breaks down and the endoplasmic reticulum and Golgi apparatus disperse to the periphery of the cell, while the mitotic spindle assembles in a central "exclusion zone" that is largely devoid of membranes and organelles. "We wanted to know what happens to misaligned chromosomes that find themselves among the organelle remnants, or endomembranes, beyond the exclusion zone," explains Stephen Royle of Warwick Medical School.

Using light and electron microscopy, Royle and colleagues observed that misaligned chromosomes beyond the exclusion zone become ensheathed by 3–4 layers of endomembranes. "That immediately raised questions about the fate of these chromosomes and whether ensheathing leads to aberrant mitosis," says first author Nuria Ferrandiz.

The researchers found that ensheathed chromosomes fail to form stable attachments to spindle microtubules, preventing their movement to the metaphase plate. As a result, cells containing ensheathed chromosomes activate the spindle assembly checkpoint, delaying their progression through mitosis. Eventually, however, the checkpoint is exhausted, and the cells divide without aligning the ensheathed chromosome correctly, increasing the frequency of chromosome missegregation and micronucleus formation. Notably, the nuclear envelope surrounding these micronuclei was disrupted, exposing the missegregated chromosomes to the cytosol and leaving them potentially vulnerable to DNA damage.

To confirm that ensheathing causes chromosome missegregation, Royle and colleagues developed a method to clear endomembranes away from misaligned chromosomes positioned outside of the exclusion zone. In cells expressing an FKBP-tagged ER-resident membrane protein and an FRB-tagged plasma membrane protein, the researchers were able to induce the relocalization of most endomembranes to the cell boundary by adding rapamycin, a drug that promotes FKBP-FRB dimerization. Removing endomembranes in this way allowed formerly ensheathed chromosomes to move to the metaphase plate and segregate correctly.

"Taken together, our findings indicate that endomembranes are a risk factor for chromosome missegregation if misaligned chromosomes go beyond the exclusion zone boundary during mitosis," Royle says.

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## ORIGINAL PAPER

Ferrandiz, N., L. Downie, G.P. Starling, and S.J. Royle. 2022. Endomembranes promote chromosome missegregation by ensheathing misaligned chromosomes. *J. Cell Biol.* 221 (6): e202203021.

https://doi.org/10.1083/jcb.202203021

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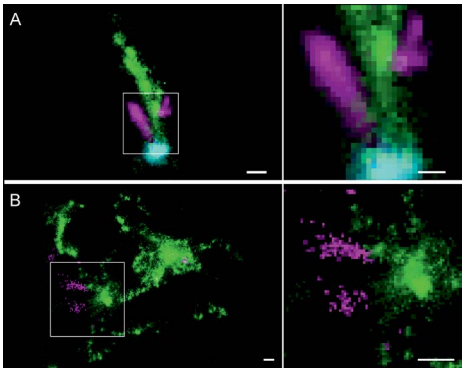
# VISUALIZING mRNA ESCAPE FROM RECYCLING ENDOSOMES

***Single-molecule imaging study reveals that mRNAs taken up into cells by endocytosis are released into the cytoplasm from endosomal recycling tubules, suggesting ways to optimize RNA therapeutics.***




Examples of single-molecule localization microscopy showing (A) a concentrated mRNA signal (magenta) representing a LNP-mRNA complex at the tip of a recycling tubule containing transferrin (green) and EGF (cyan), and (B) a dispersed mRNA signal in the cytoplasm after mRNA escape. 

In addition to the development of mRNA-based vaccines, recent years have seen increasing interest in the therapeutic use of RNAs to inhibit gene expression or drive protein production. RNAs can be delivered into cells by packaging them in lipid nanoparticles (LNPs), which associate with the low-density lipoprotein receptor and undergo receptor-mediated endocytosis. But LNPs with different chemical compositions can vary widely in their ability to deliver RNAs without any toxic side effects to the cell. Understanding these differences—and designing better delivery systems—is complicated by the fact that it remains unclear where and how RNAs escape the endosomal lumen and enter the cytoplasm, where they can have their therapeutic effect.

"We wanted to determine whether differences in LNP-mediated mRNA delivery efficacy may originate from variations in uptake and/or transport to endosomal subcompartments with higher probability to escape than others," says Marino Zerial from the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden.

Zerial and colleagues, including co-first authors Prasath Paramasivam and Christian Franke compared the ability of six different LNP formulations to deliver mRNAs encoding eGFP into cells. Surprisingly, the efficacy of RNA delivery into the cytoplasm—as assessed by eGFP expression—did not completely correlate with the degree of uptake into cells.

Instead, the researchers found, the efficacy of mRNA delivery can be predicted by the degree to which, after been internalized, LNP–mRNA complexes enter early/recycling endosomes. Conventional confocal microscopy techniques do not have sufficient resolution to determine whether a molecule is inside or outside an endosome, making endosomal escape difficult to follow. Using single-molecule localization microscopy, Zerial and colleagues were able to resolve individual LNPs within early endosomes, follow their unpacking, and even detect single mRNA molecules in the cytoplasm next to endosomal recycling tubules, presumably having escaped across the endosomal membrane. The researchers speculate that the high membrane curvature of recycling tubules may allow LNP-derived lipids to destabilize their membranes, facilitating the escape of mRNAs into the cytoplasm.

In contrast, some LNP-mRNAs accumulate inside enlarged early endosomes that fail to acidify and mature into late endosomes. mRNAs appear to be unable to escape from these endosomes, and the resulting blockade in cargo transport and degradation may contribute to cellular toxicity.

"Our results define quantitative endosomal parameters that can guide the development of new mRNA formulations toward high efficacy and low cytotoxicity by optimizing not only the uptake of LNPs into cells but also the subsequent steps that lead to mRNA escape into the cytoplasm," Zerial says. "A possible strategy would be to develop LNPs that can distribute more evenly between endosomes or even preferentially sort to recycling tubules."

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## ORIGINAL PAPER

Paramasivam, P., C. Franke, M. Stöter, A. Höijer, S. Bartesaghi, A. Sabirsh, L. Lindfors, M.Y. Arteta, A. Dahlén, A. Bak, S. Andersson, Y. Kalaidzidis, M. Bickle, and M. Zerial. 2022. Endosomal escape of delivered mRNA from endosomal recycling tubules visualized at the nanoscale. *J. Cell Biol.* 221 (2): e202110137.

https://doi.org/10.1083/jcb.202110137

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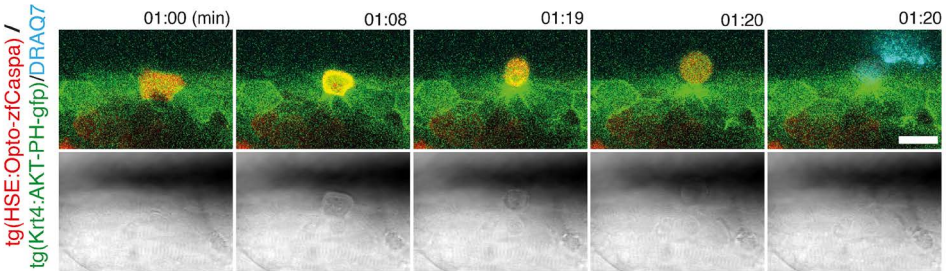
# OPTOGENETIC ACTIVATORS OF PROGRAMMED CELL DEATH

***Researchers develop a set of tools to specifically induce either apoptosis, necroptosis, or pyroptosis with high spatiotemporal precision.***

There are multiple programmed cell death (PCD) pathways that eliminate unnecessary or diseased cells during development and tissue homeostasis. The different pathways are initiated by different intrinsic and extrinsic signals that lead to the activation of different effector proteins that induce cell death in different ways.

"However, while the pathways and checkpoints that control PCD are well understood, comparably little is known about how the distinct forms of PCD differ in their outcome for the dying cell, its neighbors, and the organism as a whole," explains Petr Broz of the University of Lausanne. This is due, in part, to a lack of experimental methods that can activate specific PCD pathways in individual cells at specific timepoints.

Broz and colleagues, including first author Kateryna Shkarina therefore developed a set of tools that enable the light-mediated induction of three distinct PCD pathways: apoptosis, necroptosis, and pyroptosis. These optogenetically controlled cell death effectors (optoCDEs) consist of an *Arabidopsis*-derived photosensitive protein that rapidly oligomerizes in response to blue light fused to specific effectors for each PCD pathway. These effectors included the pyroptosis-inducing protease caspase-1, the necroptosis-inducing kinase RIPK3 and pseudokinase MLKL, and the apoptosis-inducers caspase-8 and caspase-9.




In the skin of a zebrafish larva, photoactivation induces a cell expressing the optogenetic pyroptosis effector opto-zfCaspa (red) to be extruded from a layer of keratinocytes (green) and undergo cell lysis (blue staining). © 2022 Shkarina et al.

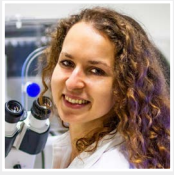
The optoCDEs allow specific PCD pathways to be rapidly activated in single cells within 2D epithelia, 3D organoids, and even live animals such as zebrafish larvae. "To highlight this application, we studied the differential fates of individual epithelial cells upon apoptosis, necroptosis, or pyroptosis induction," says Shkarina.

The researchers found that Caco-2 cells undergoing pyroptosis or necroptosis are quickly extruded from the epithelial monolayer, whereas cells undergoing apoptosis fragment into apoptotic bodies that are engulfed by neighboring cells in a process known as efferocytosis. Broz and colleagues were able to visualize the actin cytoskeleton rearrangements required for both extrusion and efferocytosis, and determined that both processes are dependent on sphingosine-1-phosphate signaling.

Notably, the fate of dying cells appears to be cell-type specific: whereas apoptotic keratinocytes were also efferocytosed in zebrafish larvae, apoptotic MDCK or MCF10A cells were, in keeping with previous studies, extruded from the epithelial monolayer.

"OptoCDEs provide a versatile and specific approach to investigate PCD pathways at a spatiotemporal level, not only in individual cells but also in their neighbors in a multicellular setting," Broz says. "Their application might allow the identification and study of new biological processes during PCD and a better understanding of associated phenomena such as membrane repair and recovery from sublethal stimuli, cell extrusion, and migration."

## RESEARCHER DETAILS



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## ORIGINAL PAPER

Shkarina, K., E. Hasel de Carvalho, J.C. Santos, S. Ramos, M. Leptin, and Petr Broz. 2022. Optogenetic activators of apoptosis, necroptosis, and pyroptosis. *J. Cell Biol.* 221 (6): e202109038.

https://doi.org/10.1083/jcb.202109038

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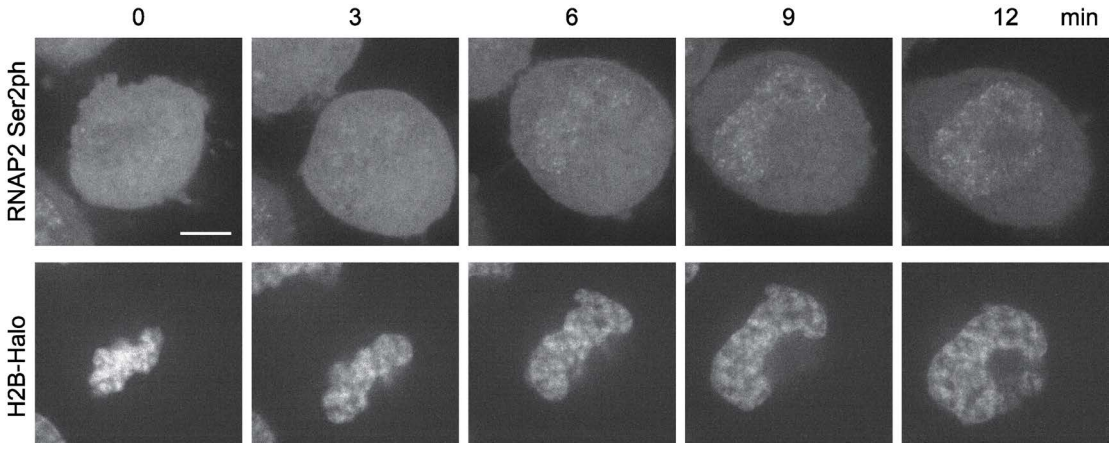
# LIVE-CELL IMAGING OF TRANSCRIPTION ELONGATION

***Researchers develop a genetically-encoded probe specific for the elongating form of RNA polymerase II.***




A time-lapse series shows the reappearance of RNAP2 Ser2ph foci (top row) as a cell co-labeled for histone H2B (bottom row) exits mitosis. © 2021 Uchino et al.

Most of the genes in eukaryotic nuclei are transcribed by RNA polymerase II (RNAP2), and this enzyme complex must be tightly regulated in order to ensure that cells express the right genes at the right time. One of the main ways in which RNAP2 is regulated is by the phosphorylation of serine residues in a long heptapeptide repeat in the C-terminal domain of the complex's catalytic subunit. For example, phosphorylation of the serine residue in the second position of the heptapeptide repeat (Ser2ph) is associated with RNAP2 engaged in transcription elongation.

"To visualize and track RNAP2 Ser2 phosphorylation in living cells, we developed a genetically encoded modification-specific intracellular antibody, or mintbody, probe," explains Hiroshi Kimura of the Tokyo Institute of Technology. "This consists of a single-chain variable fragment of an antibody that specifically recognizes RNAP2 Ser2ph, tagged with superfolder GFP."

Kimura and colleagues, including co-first authors Satoshi Uchino and Yuma Ito, found that the mintbody probe labeled numerous foci in the nuclei of transfected HeLa cells. The size and number of these foci were consistent with each of them representing a transcription "factory," where multiple RNAP2 complexes transcribe multiple genes.

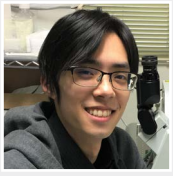
In keeping with the fact that most RNAP2-dependent transcription ceases during mitosis, Kimura and colleagues found that the mintbody-labeled foci disappeared during prophase and reappeared during early G1. The foci also disappeared when cells were treated with a kinase inhibitor that blocks RNAP2 Ser2 phosphorylation, confirming the specificity of the probe.

"We found that RNAP2 Ser2ph-mintbody foci were colocalized to a greater extent with proteins associated with transcriptional elongation compared with factors involved in transcription initiation," Kimura says.

Notably, live imaging of RNAP2 Ser2ph foci revealed that they are more mobile than sites of DNA replication, as well as foci containing the histone acetyltransferase p300, which are likely sites of enhancers.

"Based on the distinct localization and dynamics of the factors involved in transcription initiation from RNAP2 Ser2ph, it is possible that initiating and elongating RNAP2 complexes are organized differently in space and have different effects on chromosome mobility," Kimura says. "We anticipate that the RNAP2 Ser2ph mintbody will help address the question of whether new initiation events occur at or distal to pre-existing elongation RNAP2 foci."

## RESEARCHER DETAILS



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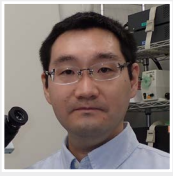

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## ORIGINAL PAPER

Uchino, S., Y. Ito, Y. Sato, T. Handa, Y. Ohkawa, M. Tokunaga, and H. Kimura. 2022. Live imaging of transcription sites using an elongating RNA polymerase II–specific probe. *J. Cell Biol.* 221 (2): e202104134.

https://doi.org/10.1083/jcb.202104134

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# A CRYO-ET SURVEY OF THE MAMMALIAN AXOPLASM

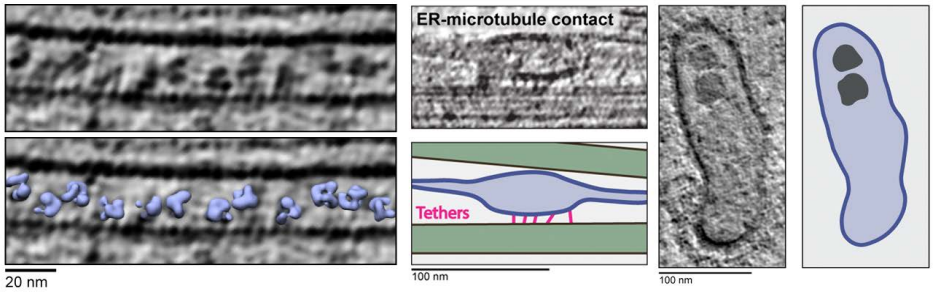
***An ultrastructural study reveals surprising new features of the cytoskeleton and intracellular compartments in mouse sensory axons.***

Neuronal axons are packed with cytoskeletal filaments, as well as organelles and protein assemblies that are being transported to the synaptic terminals or recycled back to the neuronal cell body. These intracellular structures have been closely studied for many decades, but recent developments in cryo-electron tomography (cryo-ET) are enabling researchers to examine cellular ultrastructure in more detail than ever before.

"Cryo-ET can provide subnanometer-resolution structural information in situ," explains Helen Foster, a former PhD student in Andrew Carter's group at the MRC Laboratory of Molecular Biology, Cambridge, UK. "Previous descriptions of axonal ultrastructure using cryo-ET mainly focused on synaptic regions. In our study, we characterized the axon shaft."

Foster, together with fellow PhD student Camilla Ventura Santos, cultured mouse dorsal root ganglion sensory neurons on EM grids and acquired tomograms of their axonal processes. The researchers observed numerous cytoskeletal filaments that all ran roughly parallel with each other along the axon shaft. These filaments included microtubules, actin, and intermediate filaments, but Foster and colleagues also saw a fourth class of filaments, thinner and shorter than the others, whose identity remains to be determined.

As expected, the vast majority of microtubules showed the same polarity as each other, with their stable minus ends oriented toward the cell body and their more dynamic plus ends pointed to the axon terminals. Surprisingly, however, Foster and colleagues found that the minus and plus ends of axonal microtubules have similar morphologies to each other. The g-tubulin ring complex, which nucleates axonal microtubules, does not appear to remain bound to the minus ends.




Among other new features, cryo-ET reveals ring-like MIPs in the lumen of axonal microtubules (left), tethers connecting the smooth ER to microtubules (center), and the presence of granules within membrane-bound vesicles (right). © 2021 MRC Laboratory of Molecular Biology

The researchers saw numerous globular particles, known as microtubule inner proteins (MIPs), in the lumen of axonal microtubules. These particles, which are thought to promote microtubule stability, had a defined, ring-like structure, even though they likely contain a disordered protein called MAP6.

The smooth ER forms a network of tubules throughout the axoplasm to facilitate lipid metabolism and calcium homeostasis. Foster and colleagues found that the ER tubules are connected to axonal microtubules by multiple, short tethers of unknown identity.

The researchers saw many other types of membrane-bound organelles associated with microtubules. Some of these organelles, which are transported along the microtubules by molecular motors, contained unexpected content in their lumens, such as granules and membrane sheets. Finally, Foster and colleagues also saw non-membrane-bound compartments, including known large ribonucleoprotein complexes called vaults and virus-like capsid particles of unknown identity and function.

"Our work demonstrates that cryo-ET can uncover new features even in a well-studied system like neuronal axons," says Carter. "It also presents the challenge of identifying the components that make up these novel structures."

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## ORIGINAL PAPER

Foster, H.E., C. Ventura Santos, and A.P. Carter. 2022. A cryo-ET survey of microtubules and intracellular compartments in mammalian axons. *J. Cell Biol.* 221 (2): e202103154.

https://doi.org/10.1083/jcb.202103154

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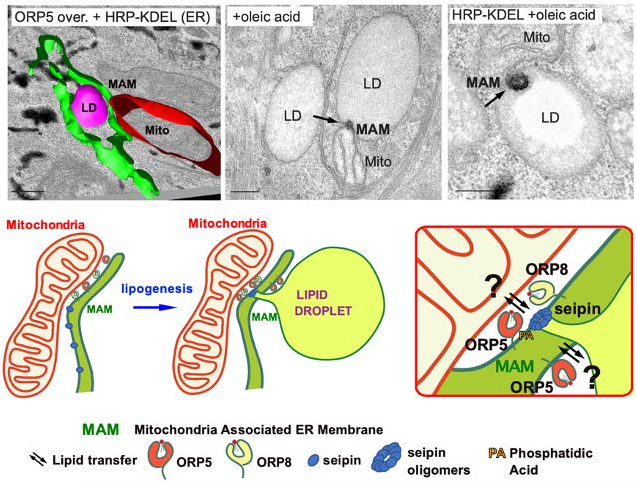
# ORP5 AND ORP8 ARE NEW PLAYERS IN LIPID DROPLET FORMATION

***Researchers show that ORP5 and ORP8 are involved in biogenesis and maintenance of lipid droplets at sites where the ER and mitochondria meet.***




EM images of mitochondria, MAMs, and nascent LDs, accompanied by a model of ORP5 and ORP8's function in LD biogenesis at these membrane contact sites.


Lipid droplets (LDs) are the primary site of lipid storage within a cell and they arise from the ER. The ER protein seipin controls their nucleation and growth, but how the formation of new LDs is regulated isn't well understood.

The teams of Abdou Rachid Thiam, Francesca Giordano, and colleagues wanted to investigate the role of ORP5 and ORP8, which belong to a family of lipid transfer proteins, are anchored to the ER and are found at ER–plasma membrane contact sites and mitochondria-associated ER membrane (MAM) subdomains.

The two teams used fluorescent microscopy to track ORP5/8, mitochondria, ER, and LDs. They found that almost all the ER subdomains in contact with LDs, where ORP5 localized, were closely associated with mitochondria. Similarly, they found that ORP8 localized to LDs that were in contact with MAM subdomains.

Depleting ORP5 and ORP8 by RNAi in HeLa cells impaired the formation of LDs. When the researchers disrupted ER–mitochondria contacts through PTPIP51 overexpression, they found a decrease in the number of nascent LDs, indicating these sites are required for LD formation. Electron microscopy analysis revealed the morphological features of the MAM subdomains where LDs arise and a specific effect of ORP5/8 knockdown on the biogenesis of LDs at MAMs.

Since seipin plays a role in where LDs form, the team tested whether it also localizes to MAMs to facilitate LD formation. In HeLa cells imaged with confocal and electron microscopy, they found that while seipin does localize with a subset of MAMs, ORP5 overexpression increases the localization of seipin to MAM–LD and ER–LD contacts. In protein binding experiments, they found that ORP5 binds to seipin, suggesting a direct interaction. Furthermore, ORP5 knockdown led to a decrease in seipin clusters at MAM–LD, which could be rescued by ORP5 overexpression. And when ER–mitochondria contact integrity was disrupted, seipin localization at MAM–LD was greatly reduced, and could not be rescued by ORP5 overexpression, indicating that ER–mitochondria contacts are required for ORP5-mediated recruitment of seipin.

These data identify ORP5 and ORP8 as novel critical players in LD biogenesis by regulating seipin targeting at MAMs. "Our study uncovers an unprecedented role of ORP5 and ORP8 in orchestrating LD biogenesis at MAMs," said the authors. They point out that ORP5/8, seipin, and MAMs play a crucial role in cellular lipid and calcium regulation—cellular functions that are dysregulated in lipodystrophies and neuronal disorders.

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## ORIGINAL PAPER

Guyard, V., V.F. Monteiro-Cardoso, M. Omrane, C. Sauvanet, A. Houcine, C. Boulogne, K.B. Mbarek, N. Vitale, O. Faklaris, N. El Khallouki, A.R. Thiam, and F. Giordano. 2022. ORP5 and ORP8 orchestrate lipid droplet biogenesis and maintenance at ER–mitochondria contact sites. *J. Cell Biol.* 221(9):e202112107.

https://doi.org/10.1083/jcb.202112107

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# WASP HELPS NEUTROPHILS SENSE SUBSTRATE TOPOLOGY

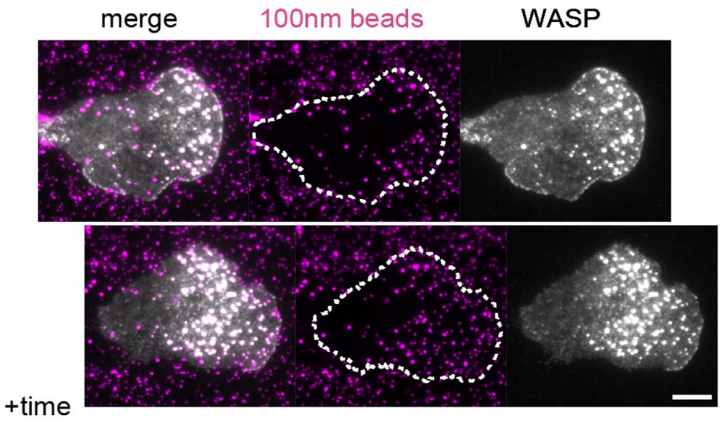
***Study reveals that the actin regulator WASP guides the 3D migration of neutrophils by coupling substrate topology and cell polarity with local actin assembly.***

The actin regulator WASP promotes the assembly of branched actin networks by activating the actin-nucleating Arp2/3 complex. Mutations in the gene encoding WASP cause Wiskott–Aldrich syndrome, an immune disorder characterized by an inability of white blood cells to migrate through tissues toward the sites of infection.

"However, despite the homing defects of immune cells in WASP-deficient patients and animal models, how WASP contributes to actin organization and immune cell migration remains poorly understood," says Orion Weiner of the University of California, San Francisco.

Weiner and colleagues, including first author Rachel Brunetti, examined the localization of endogenously expressed WASP in human neutrophils and found that the protein accumulates in numerous small puncta on the cell's ventral membrane, corresponding to sites where the underlying substrate pushes into the cell and creates membrane invaginations. Superresolution microscopy revealed that as the substrate pushes further into the cell, WASP becomes particularly enriched at the neck of the invagination.

Notably, WASP-enriched puncta are mainly located in the front half of neutrophils, gradually disappearing as the cells move over them and they become closer to the cell rear. Weiner and colleagues determined that the polarity protein Cdc42, whose activity is highest at the front of migrating neutrophils, creates a "permissive zone" in which WASP can accumulate at substrate-induced membrane invaginations.




In a neutrophil migrating over beads (magenta), WASP (grayscale) is recruited to bead-induced membrane deformations only in the front half of the cell. These WASP-enriched puncta disappear as the cell moves forward, and the beads grow closer to the cell rear. © 2021 Brunetti et al.

The researchers found that WASP recruits the Arp2/3 complex to these sites, stimulating local actin assembly that could potentially "pinch" the substrate and facilitate cell migration. Though wild-type and WASP-deficient neutrophils migrate similarly on flat surfaces, Weiner and colleagues discovered that they show distinct patterns of motility on ridged substrates that induce WASP-enriched puncta. Wild-type neutrophils tend to move in the direction of the ridges for extended distances. WASP-deficient cells, in contrast, fail to be guided along the ridges and tend to move across them instead.

"In vivo, neutrophils need to navigate complex 3D paths through dense extracellular matrices, so they have to be able to sense their physical surroundings and coordinate their movement with the features of their substrate," Weiner explains. "Our work suggests that WASP plays an essential role in this process by sensing substrate topology and selectively promoting actin polymerization around substrate-induced membrane deformations in the front half of the cell."

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## ORIGINAL PAPER

Brunetti, R.M., G. Kockelkoren, P. Raghavan, G.R.R. Bell, D. Britain, N. Puri, S.R. Collins, M.D. Leonetti, D. Stamou, and O.D. Weiner. 2022. WASP integrates substrate topology and cell polarity to guide neutrophil migration. *J. Cell Biol.* 221 (2): e202104046.

https://doi.org/10.1083/jcb.202104046

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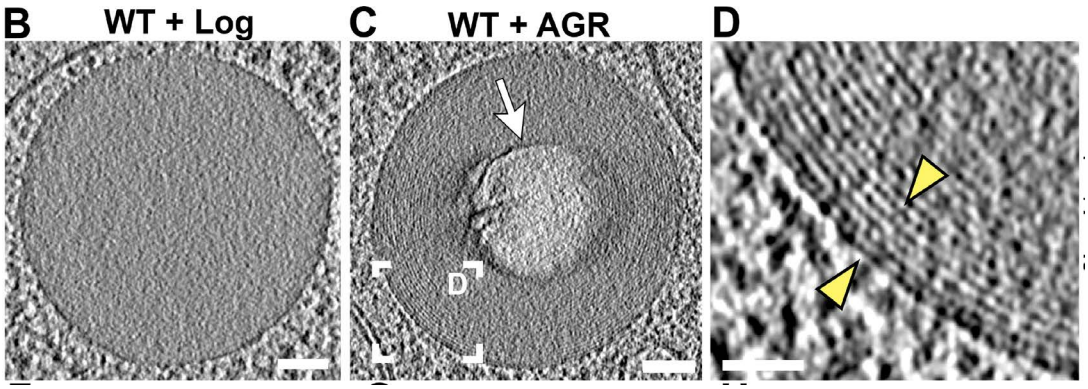
# HOW LIPIDS CAN ALTER THE LIPID DROPLET PROTEOME

***Removal of triglycerides from lipid droplets can trigger phase changes that alter the protein composition of lipid droplets.***




In yeast cell lipid droplets, visualized by cryo-ET, glucose restriction (AGR) induces phase transition of lipids in the core, which rearrange to form onion-like layers. © 2022 Rogers et al.

As well as storing energy-rich neutral fats, lipid droplets (LDs) are involved in signaling, development, and metabolism. LDs are decorated by specific proteins, but how the lipids inside the LD are organized and how they influence the composition and dynamics of the LD proteome is not well understood.

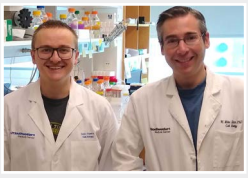
LDs are derived from the ER and are composed of a hydrophobic core of triglycerides (TGs) and sterol-esters (SEs) that are surrounded by a phospholipid monolayer. While the neutral lipids inside LDs are generally amorphous, certain stimuli can induce phase changes, in which the arrangement of lipids changes from a liquid to a solid or "gel." These phase transitions are attributed to SEs and are associated with pathologies like atherosclerosis.

Sean Rogers, Mike Henne and colleagues previously found that transferring budding yeast to a low glucose environment leads to the production of SEs, so they wanted to determine if glucose depletion impacts LDs. The team found that glucose deprivation triggers a phase transition in LDs, generating liquid crystalline lattices in the LD core. This transition is driven by the lipolysis of triglycerides, decreasing the TG:SE ratio within the LDs. Computer modeling suggested that under conditions of low TG:SE ratios, SEs and TGs can arrange in a non-uniform way, with SE becoming enriched near the phospholipid monolayer. This, in turn, disrupts phospholipid packing and could alter the recruitment of proteins to the LD surface.

In experiments measuring changes in the LD proteome, as well as imaging experiments, the team observed changes in LD protein abundance and redistribution of LD proteins upon glucose depletion. They observed an increase in proteins associated with fatty acid oxidation, which indicates that glucose restriction promotes the mobilization of TGs from LDs, providing fatty acids as fuel for yeast.

"Taken together, our data suggest that upon glucose restriction, TG lipolysis triggers spontaneous lipid demixing within LDs that supports liquid crystalline phase transitions and the subsequent remodeling of the LD surface proteome," said Rogers. "This study is a significant step toward understanding how metabolic cues influence the lipid phase transition properties of organelles, and ultimately organelle lipid and proteome composition and function."

## RESEARCHER DETAILS



Sean Rogers (left) and Mike Henne (right)

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Rogers, S., L. Gui, A. Kovalenko, V. Zoni, M. Carpentier, K. Ramji, K. Ben Mbarek, A. Bacle, P. Fuchs, P. Campomanes, E. Reetz, N. Ortiz Speer, E. Reynolds, A. Rachid Thiam, S. Vanni, D. Nicastro, and W.M. Henne. 2022. Triglyceride lipolysis triggers liquid crystalline phases in lipid droplets and alters the LD proteome. *J. Cell Biol.* 221(11):e202205053.

https://doi.org/10.1083/jcb.202205053

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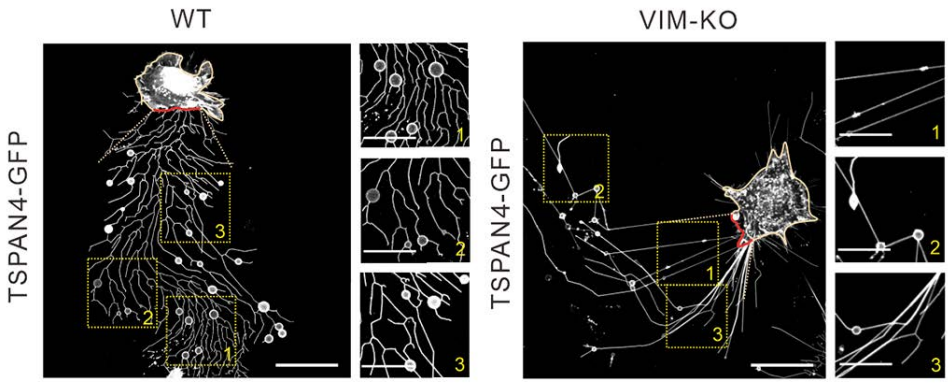
# HOW CELL MIGRATION REGULATES THE FORMATION OF MIGRASOMES

***Vesicles called migrasomes form on the trailing ends of moving cells, and new research shows that speed and direction regulate this process.***

As migrating cells move, long tubular strands, called retraction fibers (RFs), trail behind them. Vesicles grow on the tips and intersections of RFs, which eventually break, releasing the vesicles. This process was named migracytosis, and the vesicles, known as migrasomes, are thought to be important for intercellular communication, as well as removing unwanted material from the cell. But whether cell migration patterns contribute to migrasome formation was not known. Changyuan Fan, Yaming Jiu, and colleagues found that cell migration speed and direction can regulate migracytosis.




Migrasomes, visualized by migrasome membrane protein TSPAN4, are observed more frequently in WT L929 cells compared to those with vimentin (VIM) knocked out. © 2022 Fan et al.

During random migration, cells often change direction. To explore whether this has any impact on migrasomes, the team followed live fibroblast cells expressing Tetraspanin 4 (TSPAN4), a protein that's abundant in the membrane of migrasomes. They traced RFs on cells turning and going straight, and found more migrasomes on the RFs of cells persistently moving straight. They noticed that the cell rear end becomes wider and RFs subsequently increase when a cell persistently moves in a straight direction. In contrast, the rear end is longer and more narrow while turning, resulting in fewer RFs.

Faster, straight-moving cells also generated more migrasomes than slower, straight-moving ones. Faster cells had longer RFs than slower moving cells, indicating that migration speed regulates migrasome formation via the length of derived RFs.

The cytoskeletal protein vimentin is known to be involved in cell migration, but is not found in RFs, so the team wanted to determine whether depleting vimentin, and therefore changing cell migration, would lead to changes in migrasomes. They found that depletion of vimentin decreased migration persistence, increased turning frequency, and reduced the speed of cell migration. Cells without vimentin also had fewer migrasomes, suggesting that fast, directed cell migration enhances migrasome formation.

"Our data explicate the critical roles of two cell migration patterns, persistence and speed, in the control of migrasome formation by regulating retraction fibers," Jiu said. "Cell migration plays a key role in organ formation, tissue regeneration, wound healing, cancer metastasis, and many diseases," she points out, adding that "the ability of migration behavior to control migrasome formation potentiates a spatiotemporal dynamic regulatory function of migrasomes during various physiological and pathological processes."

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## ORIGINAL PAPER

Fan, C., X. Shi, K. Zhao, L. Wang, K. Shi, Y. Liu, H. Li, B. Ji, and Y. Jiu. 2022. Cell migration orchestrates migrasome formation by shaping retraction fibers. *J. Cell Biol.* 221(4):e202109168.

https://doi.org/10.1083/jcb.202109168

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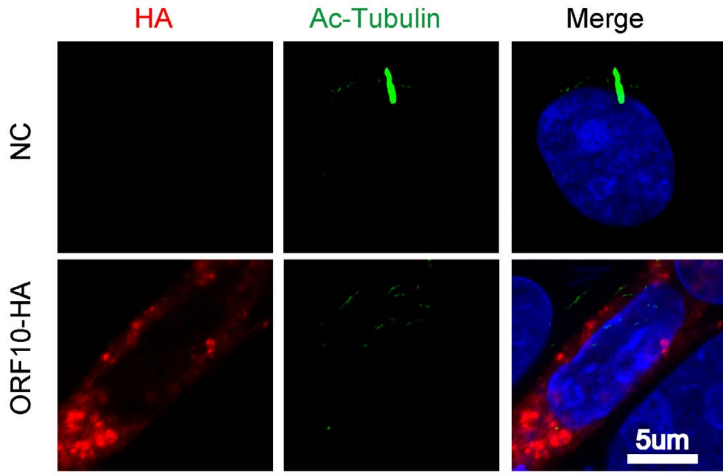
# COVID-19 SYMPTOMS MAY BE DUE TO DISRUPTIONS IN CILIA

***SARS-CoV-2 protein ORF10 can impair cilia by enhancing the degradation of ciliary proteins.***




In a lung cell line, expression of ORF10 (red) inhibits the formation of cilia (green). © 2022 Wang et al.

The first defense against inhaled pathogens, like viruses, is the cilia in our airways, which beat in a coordinated way to move mucus, and anything trapped in it, out of the airway and to our mouth. Sensory cilia in the olfactory epithelium, meanwhile, contain numerous odorant receptors involved in taste and smell. Because mucus accumulation and loss of taste and smell are common symptoms of COVID-19, Liying Wang, Wei Li, and colleagues studied whether the virus that causes COVID-19, SARS-CoV-2, can cause cilia dysregulation.

Viruses have evolved to usurp the ubiquitin system to promote the destruction of antiviral proteins produced by a cell in response to the infection. A protein encoded by the SARS-CoV-2 genome, called ORF10, was previously reported to interact with the $CUL2^{ZYG11B}$ ubiquitin ligase complex, which adds ubiquitin to other proteins and targets them for degradation.

The team confirmed that ORF10 binds to ZYG11B, a CUL2 subunit, in vitro. In proteomics experiments, they found that overexpression of ORF10 in HEK 293T cells caused the downregulation of 352 proteins and the upregulation of only two proteins, indicating that ORF10 hampers protein stability. The majority of the downregulated proteins were involved in the process of making or stabilizing cilia.

In two ciliated cell lines, the team found that expression of ORF10 led to a significant reduction in cilium assembly. Overexpression of ZYG11B also led to defects in ciliogenesis, whereas ZYG11B knockdown rescued cilia assembly in ORF10-expressing cells. Further experiments showed that one of the cilia proteins most impacted by ORF10 is IFT46, which delivers new proteins to the cilium for biogenesis and maintenance. ZYG11B overexpression also promoted IFT46 degradation, suggesting that ORF10 impairs cilia by promoting IFT46 degradation through $CUL2^{ZYG11B}$.

To simulate a SARS-CoV-2 infection, the team created a lentivirus expressing both ORF10 and the SARS-CoV-2 spike protein so that it could infect mice that express the human ACE2 receptor. In these mice, they observed a decrease in IFT46 levels and damage to the ciliary layers in the trachea. They also found a reduction in cilia in primary human nasal epithelial cells infected with their ORF10 lentivirus.

"Our data indicate that SARS-CoV-2 ORF10 interacts with ZYG11B, which stimulates the ubiquitination activity of $CUL2^{ZYG11B}$," Wang says. "Given that the ORF10–ZYG11B interaction is essential for the cilia dysfunction–related symptoms of COVID-19, some specific targeting agents that disrupt the interaction between ORF10 and ZYG11B might be considered as potential treatments."

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Wang, L., C. Liu, B. Yang, H. Zhang, J. Jiao, R. Zhang, S. Liu, S. Xiao, Y. Chen, B. Liu, Y. Ma, X. Duan, Y. Guo, M. Guo, B. Wu, X. Wang, X. Huang, H. Yang, Y. Gui, M. Fang , L. Zhang, S. Duo, X. Guo, and W. Li. 2022. SARS-CoV-2 ORF10 impairs cilia by enhancing $CUL2^{ZYG11B}$ activity. *J. Cell Biol.* 221(7):e202108015.

https://doi.org/10.1083/jcb.202108015

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