
The Year in Cell Biology 2023
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Introduction

The *Journal of Cell Biology* is pleased to present 11 articles published in the last year that most interested our readers.

From the nanoscale structure of focal adhesions to ciliary homeostasis in astrocytes, this collection exemplifies the range of topics covered by *JCB* as well as the extraordinary quality and impact that they share. These articles convey exciting new discoveries in immunology, the cell cycle, organelle contacts, and neuroscience, and include exceptional new methods that have captured the interest of our readers.

For over 65 years, the *Journal of Cell Biology* has served as a preeminent outlet for impactful findings on the structures and operations within and among cells. We are indebted to our authors and our reviewers, whose shared commitment to the highest standards of research drive the many cell biological advances reported in our journal, including those highlighted here. Finally, we thank our diverse and dedicated readers around the world. Your interest in cell biology makes *JCB* possible.

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ULTRAFAST SINGLE-MOLECULE FLUORESCENCE IMAGING

The spatial resolution of fluorescence microscopy has recently been greatly enhanced. However, improvements in temporal resolution have been limited, despite their importance for examining living cells.

We developed an ultrafast camera system that enables the highest time resolutions in single fluorescent-molecule imaging to date, which were photon limited by fluorophore photophysics: 33 and 100 µs with single-molecule localization precisions of 34 and 20 nm, respectively, for Cy3, the optimal fluorophore we identified. Using theoretical frameworks developed for the analysis of single-molecule trajectories in the plasma membrane (PM), this camera successfully detected fast hop diffusion of membrane molecules in the PM, previously detectable only in the apical PM using less preferable 40-nm gold probes, thus helping to elucidate the principles governing PM organization and molecular dynamics.

Furthermore, as described in the companion paper (below), this camera allows simultaneous data acquisitions for PALM/dSTORM at as fast as 1 kHz, with 29/19 nm localization precisions in the 640 × 640 pixel view-field.

ORIGINAL PAPER

Fujiwara, T.K., S. Takeuchi, Z. Kalay, Y. Nagai, T.A. Tsunoyama, T. Kalkbrenner, K. Iwasawa, K.P. Ritchie, K.G.N. Suzuki, and A. Kusumi. 2023. Development of ultrafast camera-based single fluorescent-molecule imaging for cell biology. *J. Cell Biol.* 222 (8): e202110160. https://doi.org/10.1083/jcb.202110160

DYNAMIC NANO-ARCHITECTURE OF FOCAL ADHESIONS

Using our newly developed ultrafast camera described in the companion paper (above), we reduced the data acquisition periods required for photoactivation/photoconversion localization microscopy (PALM, using mEos3.2) and direct stochastic reconstruction microscopy (dSTORM, using HMSiR) by a factor of ≈30 compared with standard methods, for much greater view-fields, with localization precisions of 29 and 19 nm, respectively, thus opening up previously inaccessible spatiotemporal scales to cell biology research.

Simultaneous two-color PALM-dSTORM and PALM-ultrafast (10 kHz) single fluorescent-molecule imaging-tracking has been realized. They revealed the dynamic nanoorganization of the focal adhesion (FA), leading to the compartmentalized archipelago FA model, consisting of FA-protein islands with broad diversities in size (13–100 nm; mean island diameter ≈30 nm), protein copy numbers, compositions, and stoichiometries, which dot the partitioned fluid membrane (74-nm compartments in the FA vs. 109-nm compartments outside the FA). Integrins are recruited to these islands by hop diffusion. The FA-protein islands form loose ≈320 nm clusters and function as units for recruiting FA proteins.

ORIGINAL PAPER

Fujiwara, T.K., T.A. Tsunoyama, S. Takeuchi, Z. Kalay, Y. Nagai, T. Kalkbrenner, Y.L. Nemoto, L.H. Chen, A.C.E. Shibata, K. Iwasawa, K.P. Ritchie, K.G.N. Suzuki, and A. Kusumi. 2023. Ultrafast single-molecule imaging reveals focal adhesion nano-architecture and molecular dynamics. *J. Cell Biol.* 222 (8): e202110162. https://doi.org/10.1083/jcb.202110162

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A PI(3,5)P_2 PROBE REVEALS DISTINCT DYNAMICS IN DIFFERENT ENDOCYTIC PATHWAYS

Phosphoinositide signaling lipids (PIPs) are key regulators of membrane identity and trafficking. Of these, PI(3,5)P_2 is one of the least well-understood, despite key roles in many endocytic pathways including phagocytosis and macropinocytosis. PI(3,5)P_2 is generated by the phosphoinositide 5-kinase PIKfyve, which is critical for phagosomal digestion and antimicrobial activity. However, PI(3,5)P_2 dynamics and regulation remain unclear due to a lack of reliable reporters.

Using the amoeba *Dictyostelium discoideum*, we identify SnxA as a highly selective PI(3,5)P_2-binding protein and characterize its use as a reporter for PI(3,5)P_2 in both *Dictyostelium* and mammalian cells. Using GFP-SnxA, we demonstrate that *Dictyostelium* phagosomes and macropinosomes accumulate PI(3,5)P_2 3 min after engulfment but are then retained differently, indicating pathway-specific regulation.

We further find that PIKfyve recruitment and activity are separable and that PIKfyve activation stimulates its own dissociation. SnxA is therefore a new tool for reporting PI(3,5)P_2 in live cells that reveals key mechanistic details of the role and regulation of PIKfyve/PI(3,5)P_2.

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Vines, J.H., H. Maib, C.M. Buckley, A. Gueho, Z. Zhu, T. Soldati, D.H. Murray, and J.S. King. 2023. A PI(3,5)P_2 reporter reveals PIKfyve activity and dynamics on macropinosomes and phagosomes. *J. Cell Biol.* 222 (9): e202209077. https://doi.org/10.1083/jcb.202209077

THE CELL CYCLE CONTROLS EPIDERMAL CALCIUM SIGNALING

Skin homeostasis is maintained by stem cells, which must communicate to balance their regenerative behaviors. Yet, how adult stem cells signal across regenerative tissue remains unknown due to challenges in studying signaling dynamics in live mice.

We combined live imaging in the mouse basal stem cell layer with machine learning tools to analyze patterns of Ca^{2+} signaling. We show that basal cells display dynamic intercellular Ca^{2+} signaling among local neighborhoods. We find that these Ca^{2+} signals are coordinated across thousands of cells and that this coordination is an emergent property of the stem cell layer. We demonstrate that G2 cells are required to initiate normal levels of Ca^{2+} signaling, while connexin43 connects basal cells to orchestrate tissue-wide coordination of Ca^{2+} signaling. Lastly, we find that Ca^{2+} signaling drives cell cycle progression, revealing a communication feedback loop.

This work provides resolution into how stem cells at different cell cycle stages coordinate tissue-wide signaling during epidermal regeneration.

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Moore, J.L., D. Bhaskar, F. Gao, C. Matte-Martone, S. Du, E. Lathrop, S. Ganesan, L. Shao, R. Norris, N. Campamà Sanz, K. Annusver, M. Kasper, A. Cox, C. Hendry, B. Rieck, S. Krishnaswamy, and V. Greco. 2023. Cell cycle controls long-range calcium signaling in the regenerating epidermis. *J. Cell Biol.* 222 (7): e202302095. https://doi.org/10.1083/jcb.202302095

GIANT ESCRT STRUCTURES SURROUND CELL ADHESION SITES

Endosomal Sorting Complex Required for Transport (ESCRT) proteins can be transiently recruited to the plasma membrane for membrane repair and formation of extracellular vesicles. We discovered micrometer-sized worm-shaped ESCRT structures that stably persist for multiple hours at the plasma membrane of macrophages, dendritic cells, and fibroblasts.

These structures surround clusters of integrins and known cargoes of extracellular vesicles. The ESCRT structures are tightly connected to the cellular support and are left behind by the cells together with surrounding patches of membrane. The phospholipid composition is altered at the position of the ESCRT structures, and the actin cytoskeleton is locally degraded, which are hallmarks of membrane damage and extracellular vesicle formation. Disruption of actin polymerization increased the formation of the ESCRT structures and cell adhesion. The ESCRT structures were also present at plasma membrane contact sites with membrane-disrupting silica crystals.

We propose that the ESCRT proteins are recruited to adhesion-induced membrane tears to induce extracellular shedding of the damaged membrane.

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Stempels, F.C., M. Jiang, H.M. Warner, M.-L. Moser, M.H. Janssens, S. Maassen, I.H. Nelen, R. de Boer, W.F. Jiemy, D. Knight, J. Selley, R. O'Cualain, M.V. Baranov, T.C.Q. Burgers, R. Sansevrino, D. Milovanovic, P. Heeringa, M.C. Jones, R. Vlijm, M. ter Beest, and G. van den Bogaart. 2023. Giant worm-shaped ESCRT scaffolds surround actin-independent integrin clusters. *J. Cell Biol.* 222 (7): e202205130. https://doi.org/10.1083/jcb.202205130

DISTINCT ACTIN NANOSTRUCTURES AT PRESYNAPSES

The architecture of the actin cytoskeleton that concentrates at presynapses remains poorly known, hindering our understanding of its roles in synaptic physiology. In this work, we measure and visualize presynaptic actin by diffraction-limited and super-resolution microscopy, thanks to a validated model of bead-induced presynapses in cultured neurons.

We identify a major population of actin-enriched presynapses that concentrates more presynaptic components and shows higher synaptic vesicle cycling than their non-enriched counterparts. Pharmacological perturbations point to an optimal actin amount and the presence of distinct actin structures within presynapses. We directly visualize these nanostructures using Single Molecule Localization Microscopy, defining three distinct types: an actin mesh at the active zone, actin rails between the active zone and deeper reserve pools, and actin corrals around the whole presynaptic compartment.

Finally, CRISPR tagging of endogenous actin allows us to validate our results in natural synapses between cultured neurons, confirming the role of actin enrichment and the presence of three types of presynaptic actin nanostructures.

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AN E-SYT1-PERK COMPLEX TRANSFERS LIPIDS FROM THE ER TO MITOCHONDRIA

The integrity of ER–mitochondria appositions ensures transfer of ions and phospholipids (PLs) between these organelles and exerts crucial effects on mitochondrial bioenergetics. Malfunctions within the ER–mitochondria contacts altering lipid trafficking homeostasis manifest in diverse pathologies, but the molecular effectors governing this process remain ill-defined.

We report that PERK promotes lipid trafficking at the ER–mitochondria contact sites (EMCS) through a non-conventional, unfolded protein response-independent mechanism. PERK operates as an adaptor for the recruitment of the ER–plasma membrane tether and lipid transfer protein (LTP) Extended-Synaptotagmin 1 (E-Syt1) within the EMCS. In resting cells, the heterotypic E-Syt1-PERK interaction endorses transfer of PLs between the ER and mitochondria. Weakening the E-Syt1-PERK interaction or removing the lipid transfer SMP-domain of E-Syt1 compromises mitochondrial respiration.

Our findings unravel E-Syt1 as a PERK-interacting LTP and molecular component of the lipid trafficking machinery of the EMCS, which critically maintains mitochondrial homeostasis and fitness.

ORIGINAL PAPER

Sassano, M.L., A.R. van Vliet, E. Vervoort, S. Van Eygen, C. Van den Haute, B. Pavie, J. Roels, J.V. Swinnen, M. Spinazzi, L. Moens, K. Casteels, I. Meyts, P. Pinton, S. Marchi, L. Rochin, F. Giordano, B. Felipe-Abrio, and P. Agostinis. 2023. PERK recruits E-Syt1 at ER–mitochondria contacts for mitochondrial lipid transport and respiration. *J. Cell Biol.* 222 (3): e202206008. https://doi.org/10.1083/jcb.202206008

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HIV INFECTS MACROPHAGES BY CELL FUSION

Macrophages are essential for HIV-1 pathogenesis and represent major viral reservoirs. Therefore, it is critical to understand macrophage infection, especially in tissue macrophages, which are widely infected in vivo, but poorly permissive to cell-free infection. Although cell-to-cell transmission of HIV-1 is a determinant mode of macrophage infection in vivo, how HIV-1 transfers toward macrophages remains elusive.

We demonstrate that fusion of infected CD4$^+$ T lymphocytes with human macrophages leads to their efficient and productive infection. Importantly, several tissue macrophage populations undergo this heterotypic cell fusion, including synovial, placental, lung alveolar, and tonsil macrophages. We also find that this mode of infection is modulated by the macrophage polarization state. This fusion process engages a specific short-lived adhesion structure and is controlled by the CD81 tetraspanin, which activates RhoA/ROCK-dependent actomyosin contractility in macrophages.

Our study provides important insights into the mechanisms underlying infection of tissue-resident macrophages and establishment of persistent cellular reservoirs in patients.

ORIGINAL PAPER

Mascarau, R., M. Woottum, L. Fromont, R. Gence, V. Cantaloube-Ferrieu, Z. Vahlas, K. Lévêque, F. Bertrand, T. Beunon, A. Métais, H. El Costa, N. Jabrane-Ferrat, Y. Gallois, N. Guibert, J.-L. Davignon, G. Favre, I. Maridonneau-Parini, R. Poincloux, B. Lagane, S. Bénichou, B. Raynaud-Messina, and C. Vérollet. 2023. Productive HIV-1 infection of tissue macrophages by fusion with infected CD4$^+$ T cells. *J. Cell Biol.* 222 (5): e202205103. https://doi.org/10.1083/jcb.202205103

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MITOCHONDRIAL DYSFUNCTION COMPROMISES ASTROCYTE CILIA

Astrocytes, often considered as secondary responders to neurodegeneration, are emerging as primary drivers of brain disease. We show that mitochondrial DNA depletion in astrocytes affects their primary cilium, the signaling organelle of a cell.

The progressive oxidative phosphorylation deficiency in astrocytes lacking mitochondrial DNA induces FOXJ1 and RFX transcription factors, known as master regulators of motile ciliogenesis. Consequently, a robust gene expression program involving motile cilia components and multiciliated cell differentiation factors are induced. While the affected astrocytes still retain a single cilium, these organelles elongate and become remarkably distorted. The data suggest that chronic activation of the mitochondrial integrated stress response (ISRmt) in astrocytes drives anabolic metabolism and promotes ciliary elongation.

Collectively, our evidence indicates that an active signaling axis involving mitochondria and primary cilia exists and that ciliary signaling is part of ISRmt in astrocytes. We propose that metabolic ciliopathy is a novel pathomechanism for mitochondria-related neurodegenerative diseases.

ORIGINAL PAPER

Ignatenko, O., S. Malinen, S. Rybas, H. Vihinen, J. Nikkanen, A. Kononov, E.S. Jokitalo, G. Ince-Dunn, and A. Suomalainen. 2023. Mitochondrial dysfunction compromises ciliary homeostasis in astrocytes. *J. Cell Biol.* 222 (1): e202203019. https://doi.org/10.1083/jcb.202203019

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REAL-TIME IMAGING OF rRNA SYNTHESIS

RNA polymerase I (Pol I) synthesizes about 60% of cellular RNA by transcribing multiple copies of the ribosomal RNA gene (rDNA). The transcriptional activity of Pol I controls the level of ribosome biogenesis and cell growth. However, there is currently a lack of methods for monitoring Pol I activity in real time.

We developed LiveArt (live imaging-based analysis of rDNA transcription) to visualize and quantify the spatiotemporal dynamics of endogenous ribosomal RNA (rRNA) synthesis. LiveArt reveals mitotic silencing and reactivation of rDNA transcription, as well as the transcriptional kinetics of interphase rDNA. Using LiveArt, we identify SRFBP1 as a potential regulator of rRNA synthesis. We show that rDNA transcription occurs in bursts and can be altered by modulating burst duration and amplitude.

Importantly, LiveArt is highly effective in the screening application for anticancer drugs targeting Pol I transcription. These approaches pave the way for a deeper understanding of the mechanisms underlying nucleolar functions.

ORIGINAL PAPER

Fu, Y., Y. Liu, T. Wen, J. Fang, Y. Chen, Z. Zhou, X. Gu, H. Wu, J. Sheng, Z. Xu, W. Zou, and B. Chen. 2023. Real-time imaging of RNA polymerase I activity in living human cells. *J. Cell Biol.* 222 (1): e202202110. https://doi.org/10.1083/jcb.202202110

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ER MEMBRANE CONTACT SITES REGULATE EXOSOME SECRETION

Exosomes are endosome-derived extracellular vesicles involved in intercellular communication. They are generated as intraluminal vesicles within endosomal compartments that fuse with the plasma membrane (PM). The molecular events that generate secretory endosomes and lead to the release of exosomes are not well understood.

We identified a subclass of non-proteolytic endosomes at prelysosomal stage as the compartment of origin of CD63 positive exosomes. These compartments undergo a Rab7a/Arl8b/Rab27a GTPase cascade to fuse with the PM. Dynamic endoplasmic reticulum (ER)-late endosome (LE) membrane contact sites (MCS) through ORP1L have the distinct capacity to modulate this process by affecting LE motility, maturation state, and small GTPase association.

Thus, exosome secretion is a multi-step process regulated by GTPase switching and MCS, highlighting the ER as a new player in exosome-mediated intercellular communication.

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ORIGINAL PAPER
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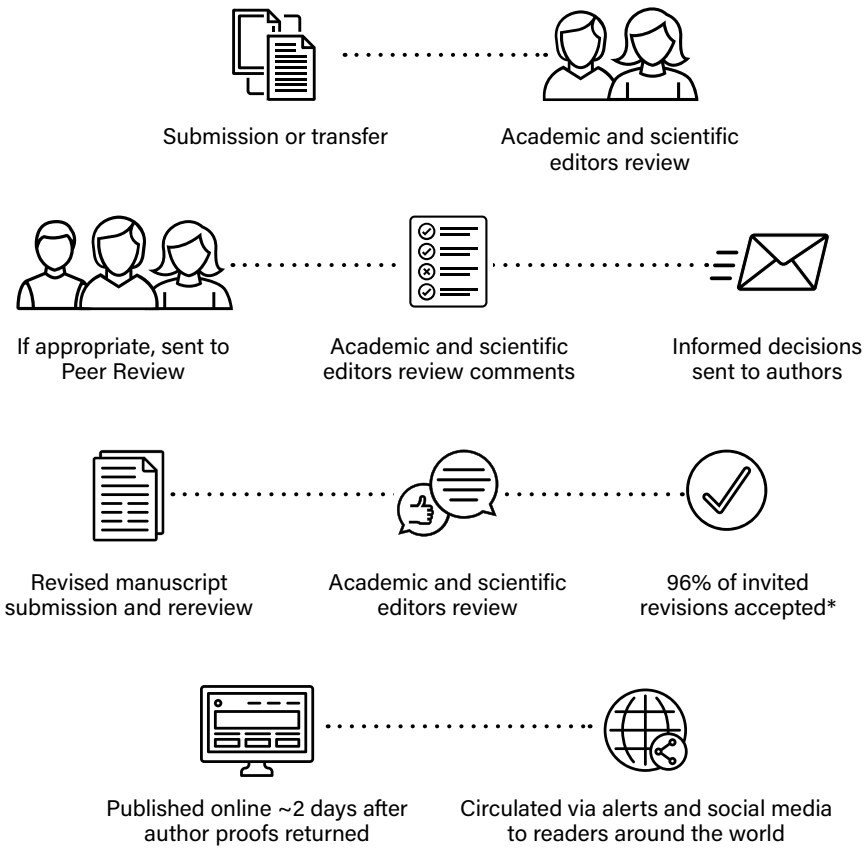

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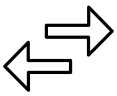

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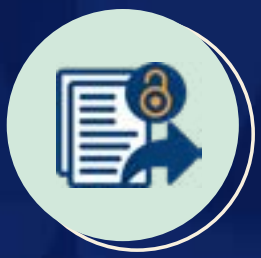

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