Année de publication : 2021

V. Kapoor, C. Carabaña (2021 Jul 6)
Cell Tracking in 3D using deep learning segmentations
scipy

Résumé

Live-cell imaging is a highly used technique to study cell migration and dynamics over time. Although many computational tools have been developed during the past years to automatically detect and track cells, they are optimized to detect cell nuclei with similar shapes and/or cells not clustering together. These existing tools are challenged when tracking fluorescently labelled membranes of cells due to cell's irregular shape, variability in size and dynamic movement across Z planes making it difficult to detect and track them. Here we introduce a detailed analysis pipeline to perform segmentation with accurate shape information, combined with BTrackmate, a customized codebase of popular ImageJ/Fiji software Trackmate, to perform cell tracking inside the tissue of interest. We developed VollSeg, a new segmentation method able to detect membrane-labelled cells with low signal-to-noise ratio and dense packing. Finally, we also created an interface in Napari, an Euler angle based viewer, to visualize the tracks along a chosen view making it possible to follow a cell along the plane of motion. Importantly, we provide a detailed protocol to implement this pipeline in a new dataset, together with the required Jupyter notebooks.

Daniel Lévy, Aurélie Di Cicco, Aurélie Bertin, Manuela Dezi (2021 Jun 7)
[Cryo-electron microcopy for a new vision of the cell and its components]
Medecine/Sciences : 379-385 : DOI : 10.1051/medsci/2021034

Résumé

Cryo-electron microscopy (cryo-EM) is a technique for imaging biological samples that plays a central role in structural biology, with high impact on research fields such as cell and developmental biology, bioinformatics, cell physics and applied mathematics. It allows the determination of structures of purified proteins within cells. This review describes the main recent advances in cryo-EM, illustrated by examples of proteins of biomedical interest, and the avenues for future development.

Eugenio de la Mora, Manuela Dezi, Aurélie Di Cicco, Joëlle Bigay, Romain Gautier, John Manzi, Joël Polidori, Daniel Castaño Díez, Bruno Mesmin, Bruno Antonny, Daniel Lévy. (2021 Jun 7)
Nanoscale architecture of a VAP-A-OSBP tethering complex at membrane contact sites
Nature Communications : DOI : 10.1038/s41467-021-23799-1

Résumé

Membrane contact sites (MCS) are subcellular regions where two organelles appose their membranes to exchange small molecules, including lipids. Structural information on how proteins form MCS is scarce. We designed an in vitro MCS with two membranes and a pair of tethering proteins suitable for cryo-tomography analysis. It includes VAP-A, an ER transmembrane protein interacting with a myriad of cytosolic proteins, and oxysterol-binding protein (OSBP), a lipid transfer protein that transports cholesterol from the ER to the trans Golgi network. We show that VAP-A is a highly flexible protein, allowing formation of MCS of variable intermembrane distance. The tethering part of OSBP contains a central, dimeric, and helical T-shape region. We propose that the molecular flexibility of VAP-A enables the recruitment of partners of different sizes within MCS of adjustable thickness, whereas the T geometry of the OSBP dimer facilitates the movement of the two lipid-transfer domains between membranes.

Linh Le, Julia Sirés-Campos, Graça Raposo, Cédric Delevoye, Michael S Marks (2021 May 22)
Melanosome biogenesis in the pigmentation of mammalian skin.
Integrative and comparative biology : DOI : icab078

Résumé

Melanins, the main pigments of the skin and hair in mammals, are synthesized within membrane-bound organelles of melanocytes called melanosomes. Melanosome structure and function are determined by a cohort of resident transmembrane proteins, many of which are expressed only in pigment cells, that localize specifically to melanosomes. Defects in the genes that encode melanosome-specific proteins or components of the machinery required for their transport in and out of melanosomes underlie various forms of ocular or oculocutaneous albinism, characterized by hypopigmentation of the hair, skin and eyes and by visual impairment. We review major components of melanosomes, including the enzymes that catalyze steps in melanin synthesis from tyrosine precursors, solute transporters that allow these enzymes to function, and structural proteins that underlie melanosome shape and melanin deposition. We then review the molecular mechanisms by which these components are biosynthetically delivered to newly forming melanosomes-many of which are shared by other cell types that generate cell type-specific lysosome-related organelles. We also highlight unanswered questions that need to be addressed by future investigation.

Ulrike Boehm, Glyn Nelson, Claire M Brown, Steve Bagley, Peter Bajcsy, Johanna Bischof, Aurelien Dauphin, Ian M Dobbie, John E Eriksson, Orestis Faklaris, Julia Fernandez-Rodriguez, Alexia Ferrand, Laurent Gelman, Ali Gheisari, Hella Hartmann, Christian Kukat, Alex Laude, Miso Mitkovski, Sebastian Munck, Alison J North, Tobias M Rasse, Ute Resch-Genger, Lucas C Schuetz, Arne Seitz, Caterina Strambio-De-Castillia, Jason R Swedlow, Roland Nitschke (2021 May 22)
QUAREP-LiMi: a community endeavor to advance quality assessment and reproducibility in light microscopy.
Nature methods : DOI : 10.1038/s41592-021-01162-y

Résumé

Silvia Benito-Martinez, Laura Salavessa, Graça Raposo, Michael S Marks, Cédric Delevoye (2021 May 22)

Melanin transfer and fate within keratinocytes in human skin pigmentation.

Integrative and comparative biology : DOI : icab094

Résumé

Human skin and hair pigmentation play important roles in social behavior but also in photoprotection from the harmful effects of ultraviolet light. The main pigments in mammalian skin, the melanins, are synthesized within specialized organelles called melanosomes in melanocytes, which sit at the basal layer of the epidermis and the hair bulb. The melanins are then transferred from melanocytes to keratinocytes, where they accumulate perinuclearly in membrane-bound organelles as a « cap » above the nucleus. The mechanism of transfer, the nature of the pigmented organelles within keratinocytes, and the mechanism governing their intracellular positioning are all debated and poorly understood, but likely play an important role in the photoprotective properties of melanin in the skin. Here, we detail our current understanding of these processes and present a guideline for future experimentation in this area.

Yolanda Gutiérrez, Sergio López-García, Argentina Lario, Silvia Gutiérrez-Eisman, Cédric Delevoye, José A Esteban (2021 May 17)

KIF13A drives AMPA receptor synaptic delivery for long-term potentiation via endosomal remodeling.

The Journal of cell biology : DOI : e202003183

Résumé

The regulated trafficking of AMPA-type glutamate receptors (AMPARs) from dendritic compartments to the synaptic membrane in response to neuronal activity is a core mechanism for long-term potentiation (LTP). However, the contribution of the microtubule cytoskeleton to this synaptic transport is still unknown. In this work, using electrophysiological, biochemical, and imaging techniques, we have found that one member of the kinesin-3 family of motor proteins, KIF13A, is specifically required for the delivery of AMPARs to the spine surface during LTP induction. Accordingly, KIF13A depletion from hippocampal slices abolishes LTP expression. We also identify the vesicular protein centaurin-α1 as part of a motor transport machinery that is engaged with KIF13A and AMPARs upon LTP induction. Finally, we determine that KIF13A is responsible for the remodeling of Rab11-FIP2 endosomal structures in the dendritic shaft during LTP. Overall, these results identify specific kinesin molecular motors and endosomal transport machinery that catalyzes the dendrite-to-synapse translocation of AMPA receptors during synaptic plasticity.

Luis Colón-Cruz, Roberto Rodriguez-Morales, Alexis Santana-Cruz, Juan Cantres-Velez, Aranza Torrado-Tapias, Sheng-Jia Lin, Guillermo Yudowski, Robert Kensler, Bruno Marie, Shawn M Burgess, Olivier Renaud, Gaurav K Varshney, Martine Behra (2021 May 7)

Cnr2 Is Important for Ribbon Synapse Maturation and Function in Hair Cells and Photoreceptors.

Frontiers in molecular neuroscience : 624265 : DOI : 10.3389/fnmol.2021.624265

Résumé

The role of the cannabinoid receptor 2 (CNR2) is still poorly described in sensory epithelia. We found strong expression in hair cells (HCs) of the inner ear and the lateral line (LL), a superficial sensory structure in fish. Next, we demonstrated that sensory synapses in HCs were severely perturbed in larvae lacking cnr2. Appearance and distribution of presynaptic ribbons and calcium channels (Ca1.3) were profoundly altered in mutant animals. Clustering of membrane-associated guanylate kinase (MAGUK) in post-synaptic densities (PSDs) was also heavily affected, suggesting a role for cnr2 for maintaining the sensory synapse. Furthermore, vesicular trafficking in HCs was strongly perturbed suggesting a retrograde action of the endocannabinoid system (ECs) via cnr2 that was modulating HC mechanotransduction. We found similar perturbations in retinal ribbon synapses. Finally, we showed that larval swimming behaviors after sound and light stimulations were significantly different in mutant animals. Thus, we propose that cnr2 is critical for the processing of sensory information in the developing larva.

Anna Fortuny, Audrey Chansard, Pierre Caron, Odile Chevallier, Olivier Leroy, Olivier Renaud, Sophie E Polo (2021 Apr 24)

Imaging the response to DNA damage in heterochromatin domains reveals core principles of heterochromatin maintenance.

Nature communications : 2428 : DOI : 10.1038/s41467-021-22575-5

Résumé

Heterochromatin is a critical chromatin compartment, whose integrity governs genome stability and cell fate transitions. How heterochromatin features, including higher-order chromatin folding and histone modifications associated with transcriptional silencing, are maintained following a genotoxic stress challenge is unknown. Here, we establish a system for targeting UV damage to pericentric heterochromatin in mammalian cells and for tracking the heterochromatin response to UV in real time. We uncover profound heterochromatin compaction changes during repair, orchestrated by the UV damage sensor DDB2, which stimulates linker histone displacement from chromatin. Despite massive heterochromatin unfolding, heterochromatin-specific histone modifications and transcriptional silencing are maintained. We unveil a central role for the methyltransferase SETDB1 in the maintenance of heterochromatic histone marks after UV. SETDB1 coordinates histone methylation with new histone deposition in damaged heterochromatin, thus protecting cells from genome instability. Our data shed light on fundamental molecular mechanisms safeguarding higher-order chromatin integrity following DNA damage.

Shanna L Bowman, Linh Le, Yueyao Zhu, Dawn C Harper, Anand Sitaram, Alexander C Theos, Elena V Sviderskaya, Dorothy C Bennett, Graça Raposo-Benedetti, David J Owen, Megan K Dennis, Michael S Marks (2021 Apr 22)

A BLOC-1-AP-3 super-complex sorts a cis-SNARE complex into endosome-derived tubular transport carriers.

The Journal of cell biology : DOI : e202005173

Résumé

Membrane transport carriers fuse with target membranes through engagement of cognate vSNAREs and tSNAREs on each membrane. How vSNAREs are sorted into transport carriers is incompletely understood. Here we show that VAMP7, the vSNARE for fusing endosome-derived tubular transport carriers with maturing melanosomes in melanocytes, is sorted into transport carriers in complex with the tSNARE component STX13. Sorting requires either recognition of VAMP7 by the AP-3δ subunit of AP-3 or of STX13 by the pallidin subunit of BLOC-1, but not both. Consequently, melanocytes expressing both AP-3δ and pallidin variants that cannot bind their respective SNARE proteins are hypopigmented and fail to sort BLOC-1-dependent cargo, STX13, or VAMP7 into transport carriers. However, SNARE binding does not influence BLOC-1 function in generating tubular transport carriers. These data reveal a novel mechanism of vSNARE sorting by recognition of redundant sorting determinants on a SNARE complex by an AP-3-BLOC-1 super-complex.

Zackie Aktary, Alejandro Conde-Perez, Florian Rambow, Mathilde Di Marco, François Amblard, Ilse Hurbain, Graça Raposo, Cédric Delevoye, Sylvie Coscoy, Lionel Larue (2021 Mar 27)

A role for Dynlt3 in melanosome movement, distribution, acidity and transfer.

Communications biology : 423 : DOI : 10.1038/s42003-021-01917-5

Résumé

Skin pigmentation is dependent on cellular processes including melanosome biogenesis, transport, maturation and transfer to keratinocytes. However, how the cells finely control these processes in space and time to ensure proper pigmentation remains unclear. Here, we show that a component of the cytoplasmic dynein complex, Dynlt3, is required for efficient melanosome transport, acidity and transfer. In Mus musculus melanocytes with decreased levels of Dynlt3, pigmented melanosomes undergo a more directional motion, leading to their peripheral location in the cell. Stage IV melanosomes are more acidic, but still heavily pigmented, resulting in a less efficient melanosome transfer. Finally, the level of Dynlt3 is dependent on β-catenin activity, revealing a function of the Wnt/β-catenin signalling pathway during melanocyte and skin pigmentation, by coupling the transport, positioning and acidity of melanosomes required for their transfer.

Sophie D Adams, Judit Csere, Gisela D'angelo, Edward P Carter, Maryse Romao, Teresa Arnandis, Martin Dodel, Hemant M Kocher, Richard Grose, Graça Raposo, Faraz Mardakheh, Susana A

Godinho (2021 Feb 16)
Centrosome amplification mediates small extracellular vesicle secretion via lysosome disruption.
Current biology : CB : 1403-1416.e7 : DOI : S0960-9822(21)00061-0

Résumé

Bidirectional communication between cells and their surrounding environment is critical in both normal and pathological settings. Extracellular vesicles (EVs), which facilitate the horizontal transfer of molecules between cells, are recognized as an important constituent of cell-cell communication. In cancer, alterations in EV secretion contribute to the growth and metastasis of tumor cells. However, the mechanisms underlying these changes remain largely unknown. Here, we show that centrosome amplification is associated with and sufficient to promote small extracellular vesicle (EV) secretion in pancreatic cancer cells. This is a direct result of lysosomal dysfunction, caused by increased reactive oxygen species (ROS) downstream of extra centrosomes. We propose that defects in lysosome function could promote multivesicular body fusion with the plasma membrane, thereby enhancing EV secretion. Furthermore, we find that EVs secreted in response to amplified centrosomes are functionally distinct and activate pancreatic stellate cells (PSCs). These activated PSCs promote the invasion of pancreatic cancer cells in heterotypic 3D cultures. We propose that EVs secreted by cancer cells with amplified centrosomes influence the bidirectional communication between the tumor cells and the surrounding stroma to promote malignancy.

Année de publication : 2020

Katia Ancelin, Yusuke Miyanari, Olivier Leroy, Maria-Elena Torres-Padilla, Edith Heard (2020 Sep 18)
Mapping of Chromosome Territories by 3D-Chromosome Painting During Early Mouse Development.
Methods in molecular biology (Clifton, N.J.) : 175-187 : DOI : 10.1007/978-1-0716-0958-3_12

Résumé

Following fertilization in mammals, the chromatin landscape inherited from the two parental genomes and the nuclear organization are extensively reprogrammed. A tight regulation of nuclear organization is important for developmental success. One main nuclear feature is the organization of the chromosomes in discrete and individual nuclear spaces known as chromosome territories (CTs). In culture cells, their arrangements can be constrained depending on their genomic content (e.g., gene density or repeats) or by specific nuclear constrains such as the periphery or the nucleolus. However, during the early steps of mouse embryonic development, much less is known, specifically regarding how and when the two parental genomes intermingle. Here, we describe a three-dimensional fluorescence in situ hybridization (3D-FISH) for chromosome painting (3D-ChromoPaint) optimized to gain understanding in nuclear organization of specific CTs following fertilization. Our approach preserves the nuclear structure, and the acquired images allow full spatial analysis of

interphase chromosome positioning and morphology across the cell cycle and during early development. This method will be useful in understanding the dynamics of chromosome repositioning during development as well as the alteration of chromosome territories upon changes in transcriptional status during key developmental steps. This protocol can be adapted to any other species or organoids in culture.

Sara El Hoss, Sylvie Cochet, Auria Godard, Hongxia Yan, Michaël Dussiot, Giacomo Frati, Bénédicte Boutonnat-Faucher, Sandrine Laurance, Olivier Renaud, Laure Joseph, Annarita Miccio, Valentine Brousse, Narla Mohandas, Wassim El Nemer (2020 Aug 29)

Fetal hemoglobin rescues ineffective erythropoiesis in sickle cell disease.

Haematologica : DOI : 10.3324/haematol.2020.265462

Résumé

While ineffective erythropoiesis has long been recognized as a key contributor to anemia in thalassemia, its role in anemia of sickle cell disease (SCD) has not been critically explored. Using in vitro and in vivo derived human erythroblasts we assessed the extent of ineffective erythropoiesis in SCD. Modeling the bone marrow hypoxic environment, we found that hypoxia induces death of sickle erythroblasts starting at the polychromatic stage, positively selecting cells with high levels of fetal hemoglobin (HbF). Cell death was associated with cytoplasmic sequestration of heat shock protein 70 and was rescued by induction of HbF synthesis. Importantly, we document that in bone marrow of SCD patients similar cell loss occurs during the final stages of terminal differentiation. Our study provides evidence for ineffective erythropoiesis in SCD and highlights an anti-apoptotic role for HbF during the terminal stages of erythroid differentiation. These findings imply that the beneficial effect on anemia of increased HbF levels is not only due to the increased life span of red cells but also a consequence of decreased ineffective erythropoiesis.

Domingues, L., I. Hurbain, F. Gilles-Marsens, J. Sirés-Campos, N. André, M. Dewulf, M. Romao, C. Viaris de Lesegno, A.S. Macé, C. Blouin, C. Guéré, K. Vié, G. Raposo, C. Lamaze, and C. Delevoye (2020 Jun 12)

Coupling of melanocyte signaling and mechanics by caveolae is required for human skin pigmentation

Nature CommunicationNature : 11 : 2988 (2020) : DOI : 10.1038/s41467-020-16738-z

Résumé

Tissue homeostasis requires regulation of cell–cell communication, which relies on signaling molecules and cell contacts. In skin epidermis, keratinocytes secrete factors transduced by melanocytes into signaling cues promoting their pigmentation and dendrite outgrowth, while melanocytes transfer melanin pigments to keratinocytes to convey skin photoprotection. How epidermal cells integrate these functions remains poorly characterized. Here, we show that caveolae are asymmetrically distributed in melanocytes and particularly abundant at the melanocyte–keratinocyte interface in epidermis. Caveolae in melanocytes are modulated

by ultraviolet radiations and keratinocytes-released factors, like miRNAs. Preventing caveolae formation in melanocytes increases melanin pigment synthesis through upregulation of cAMP signaling and decreases cell protrusions, cell–cell contacts, pigment transfer and epidermis pigmentation. Altogether, we identify that caveolae serve as molecular hubs that couple signaling outputs from keratinocytes to mechanical plasticity of pigment cells. The coordination of intercellular communication and contacts by caveolae is thus crucial to skin pigmentation and tissue homeostasis.