Year of publication 2007

Virginie Braun, Chantal Deschamps, Graça Raposo, Philippe Benaroch, Alexandre Benmerah, Philippe Chavrier, Florence Niedergang (2007 Oct 5)

AP-1 and ARF1 control endosomal dynamics at sites of FcR mediated phagocytosis.

Molecular biology of the cell : 4921-31

Summary

Phagocytosis, the mechanism of ingestion of large material and microorganisms, relies on actin polymerization and on the focal delivery of intracellular endocytic compartments. The molecular mechanisms involved in the formation and delivery of the endocytic vesicles that are recruited at sites of phagocytosis are not well characterized. Here we show that adaptor protein (AP)-1 but not AP-2 clathrin adaptor complexes are recruited early below the sites of particle attachment and are required for efficient receptor-mediated phagocytosis in murine macrophages. Clathrin, however, is not recruited with the AP complexes. We further show that the recruitment of AP-1-positive structures at sites of phagocytosis is regulated by the GTP-binding protein ARF1 but is not sensitive to brefeldin A. Furthermore, AP-1 depletion leads to increased surface levels of TNF-alpha, a cargo known to traffic through the endosomes to the plasma membrane upon stimulation of the macrophages. Together, our results support a clathrin-independent role for AP complexes in endosomal dynamics in macrophages by retaining some cargo proteins, a process important for membrane remodeling during phagocytosis.

Naomi R Stevens, Alexandre A S F Raposo, Renata Basto, Daniel St Johnston, Jordan W Raff (2007 Jun 15)

From stem cell to embryo without centrioles.

Current biology : CB : 1498-503

Summary

Centrosome asymmetry plays a key role in ensuring the asymmetric division of Drosophila neural stem cells (neuroblasts [NBs]) and male germline stem cells (GSCs) [1-3]. In both cases, one centrosome is anchored close to a specific cortical region during interphase, thus defining the orientation of the spindle during the ensuing mitosis. To test whether asymmetric centrosome behavior is a general feature of stem cells, we have studied female GSCs, which divide asymmetrically, producing another GSC and a cystoblast. The cystoblast then divides and matures into an oocyte, a process in which centrosomes exhibit a series of complex behaviors proposed to play a crucial role in oogenesis [4-6]. We show that the interphase centrosome does not define spindle orientation in female GSCs and that DSas-4 mutant GSCs [7], lacking centrioles and centrosomes, invariably divide asymmetrically to produce cystoblasts that proceed normally through oogenesis-remarkably, oocyte specification, microtubule organization, and mRNA localization are all unperturbed. Mature oocytes can be fertilized, but embryos that cannot support centriole replication arrest very

early in development. Thus, centrosomes are dispensable for oogenesis but essential for early embryogenesis. These results reveal that asymmetric centrosome behavior is not an essential feature of stem cell divisions.

Renata Basto, Jonathon Pines (2007 Apr 11)
The centrosome opens the way to mitosis.
Developmental cell : 475-7

Summary

During mitosis, the interaction between chromosomes and microtubules requires nuclear envelope disassembly in prophase. Two articles in this issue of Developmental Cell show that centrosomes have a role in promoting nuclear envelope breakdown (Hachet et al., 2007; Portier et al., 2007). Surprisingly, the role of the centrosome in this process is independent of its role as a microtubule nucleation organelle. Instead, the centrosome seems to act as a spatial regulator for the activation of the Aurora A kinase.

Renata Basto, Fanni Gergely, Viji M Draviam, Hiroyuki Ohkura, Kathryn Liley, Jordan W Raff (2007 Mar 23)
Hsp90 is required to localise cyclin B and Msps/ch-TOG to the mitotic spindle in Drosophila and humans.
Journal of cell science : 1278-87

Summary

During mitosis, cyclin B is extremely dynamic and although it is concentrated at the centrosomes and spindle microtubules (MTs) in organisms ranging from yeast to humans, the mechanisms that determine its localisation are poorly understood. To understand how cyclin B is targeted to different locations in the cell we have isolated proteins that interact with cyclin B in Drosophila embryo extracts. Here we show that cyclin B interacts with the molecular chaperone Hsp90 and with the MT-associated protein (MAP) Mini spindles (Msps; the Drosophila orthologue of XMAP215/ch-TOG). Both Hsp90 and Msps are concentrated at centrosomes and spindles, and we show that Hsp90, but not Msps, is required for the efficient localisation of cyclin B to these structures. We find that, unlike what happens with other cell cycle proteins, Hsp90 is not required to stabilise cyclin B or Msps during mitosis. Thus, we propose that Hsp90 plays a novel role in regulating the localisation of cyclin B and Msps during mitosis.

Nina Peel, Naomi R Stevens, Renata Basto, Jordan W Raff (2007 Mar 19)
Overexpressing centriole-replication proteins in vivo induces centriole overduplication and de novo formation.
Current biology : CB : 834-43

Summary

Centrosomes have important roles in many aspects of cell organization, and aberrations in their number and function are associated with various diseases, including cancer. Centrosomes consist of a pair of centrioles surrounded by a pericentriolar matrix (PCM), and their replication is tightly regulated. Here, we investigate the effects of overexpressing the three proteins known to be required for centriole replication in Drosophila-DSas-6, DSas-4, and Sak.

Year of publication 2006

Renata Basto, Joyce Lau, Tatiana Vinogradova, Alejandra Gardiol, C Geoffrey Woods, Alexey Khodjakov, Jordan W Raff (2006 Jul 4)

Flies without centrioles.

Cell : 1375-86

Summary

Centrioles and centrosomes have an important role in animal cell organization, but it is uncertain to what extent they are essential for animal development. The Drosophila protein DSas-4 is related to the human microcephaly protein CenpJ and the C. elegans centriolar protein Sas-4. We show that DSas-4 is essential for centriole replication in flies. DSas-4 mutants start to lose centrioles during embryonic development, and, by third-instar larval stages, no centrioles or centrosomes are detectable. Mitotic spindle assembly is slow in mutant cells, and approximately 30% of the asymmetric divisions of larval neuroblasts are abnormal. Nevertheless, mutant flies develop with near normal timing into morphologically normal adults. These flies, however, have no cilia or flagella and die shortly after birth because their sensory neurons lack cilia. Thus, centrioles are essential for the formation of centrosomes, cilia, and flagella, but, remarkably, they are not essential for most aspects of Drosophila development.

Year of publication 2005

Marc Dugast, Hélène Toussaint, Christelle Dousset, Philippe Benaroch (2005 Mar 8)

AP2 clathrin adaptor complex, but not AP1, controls the access of the major histocompatibility complex (MHC) class II to endosomes.

The Journal of biological chemistry : 19656-64

Summary

Newly synthesized MHC II alpha- and beta-chains associated with the invariant chain chaperone (Ii) enter the endocytic pathway for Ii degradation and loading with peptides before transport to the cell surface. It is unclear how alphabetaIi complexes are sorted from the Golgi apparatus and directed to endosomes. However, indirect evidence tends to support

direct transport involving the AP1 clathrin adaptor complex. Surprisingly, we show here that knocking down the production of AP1 by RNA interference did not affect the trafficking of alphabetali complexes. In contrast, AP2 depletion led to a large increase in surface levels of alphabetali complexes, inhibited their rapid internalization, and strongly delayed the appearance of mature MHC II in intracellular compartments. Thus, in the cell systems studied here, rapid internalization of alphabetali complexes via an AP2-dependent pathway represents a key step for MHC II delivery to endosomes and lysosomes.

Year of publication 2004

Maruxa Martinez-Campos, Renata Basto, James Baker, Maurice Kernan, Jordan W Raff (2004 Jun 9)

The Drosophila pericentrin-like protein is essential for cilia/flagella function, but appears to be dispensable for mitosis.

The Journal of cell biology : 673-83

Summary

Centrosomes consist of a pair of centrioles surrounded by an amorphous pericentriolar material (PCM). Proteins that contain a Pericentrin/AKAP450 centrosomal targeting (PACT) domain have been implicated in recruiting several proteins to the PCM. We show that the only PACT domain protein in Drosophila (the Drosophila pericentrin-like protein [D-PLP]) is associated with both the centrioles and the PCM, and is essential for the efficient centrosomal recruitment of all six PCM components that we tested. Surprisingly, however, all six PCM components are eventually recruited to centrosomes during mitosis in d-plp mutant cells, and mitosis is not dramatically perturbed. Although viable, d-plp mutant flies are severely uncoordinated, a phenotype usually associated with defects in mechanosensory neuron function. We show that the sensory cilia of these neurons are malformed and the neurons are nonfunctional in d-plp mutants. Moreover, the flagella in mutant sperm are nonmotile. Thus, D-PLP is essential for the formation of functional cilia and flagella in flies.

R D J Butcher, S Chodagam, R Basto, J G Wakefield, D S Henderson, J W Raff, W G F Whitfield (2004 Mar 5)

The Drosophila centrosome-associated protein CP190 is essential for viability but not for cell division.

Journal of cell science : 1191-9

Summary

The Drosophila CP190 and CP60 proteins interact with each other and shuttle between the nucleus in interphase and the centrosome in mitosis. Both proteins can bind directly to microtubules in vitro, and have been shown to associate with a specific pattern of loci on salivary gland polytene chromosomes, but their functions are unknown. Here we show that reducing the level of CP190 or CP60 by >90% in tissue culture cells does not significantly

interfere with centrosome or microtubule organisation, with cell division, or with cell viability. However, CP190 is an essential protein, as flies homozygous for mutations in the Cp190 gene die at late pupal stages of development. In larval brains of Cp190 mutants, mitosis is not radically perturbed, and a mutated form of CP190 (CP190DeltaM), that cannot bind to microtubules or associate with centrosomes, can rescue the lethality associated with mutations in the Cp190 gene. Thus, CP190 plays an essential role in flies that is independent of its association with centrosomes or microtubules.

Renata Basto, Frédéric Scaerou, Sarah Mische, Edward Wojcik, Christophe Lefebvre, Rui Gomes, Thomas Hays, Roger Karess (2004 Jan 9)
In vivo dynamics of the rough deal checkpoint protein during Drosophila mitosis.
Current biology : CB : 56-61

Summary

Rough Deal (Rod) and Zw10 are components of a complex required for the metazoan metaphase checkpoint and for recruitment of dynein/dynactin to the kinetochore. The Rod complex, like most classical metaphase checkpoint components, forms part of the outer domain of unattached kinetochores. Here we analyze the dynamics of a GFP-Rod chimera in living syncytial Drosophila embryos. Uniquely among checkpoint proteins, GFP-Rod robustly streams from kinetochores along microtubules, from the time of chromosome attachment until anaphase onset. Prometaphase and metaphase kinetochores continuously recruit new Rod, thus feeding the current. Rod flux from kinetochores appears to require biorientation but not tension because it continues in the presence of taxol. As with Mad2, kinetochore- and spindle-associated Rod rapidly turns over with free cytosolic Rod, both during normal mitosis and after colchicine treatment, with a t1/2 of 25-45 s. GFP-Rod coimmunoprecipitates with dynein/dynactin, and in the absence of microtubules both Rod and dynactin accumulate on kinetochores. Nevertheless, Rod and dynein/dynactin behavior are distinguishable. We propose that the Rod complex is a major component of the fibrous corona and that the recruitment of Rod during metaphase is required to replenish kinetochore dynein after checkpoint conditions have been satisfied but before anaphase onset.

Year of publication 2003

Pamela Stumptner-Cuvelette, Mabel Jouve, Julie Helft, Marc Dugast, Anne-Sophie Glouzman, Karin Jooss, Graça Raposo, Philippe Benaroch (2003 Sep 19)
Human immunodeficiency virus-1 Nef expression induces intracellular accumulation of multivesicular bodies and major histocompatibility complex class II complexes: potential role of phosphatidylinositol 3-kinase.
Molecular biology of the cell : 4857-70

Summary

Nef alters the cell surface expression of several immunoreceptors, which may contribute to viral escape. We show that Nef modifies major histocompatibility complex class II (MHC II) intracellular trafficking and thereby its function. In the presence of Nef, mature, peptide-loaded MHC II were down-modulated at the cell surface and accumulated intracellularly, whereas immature (invariant [Ii] chain-associated) MHC II expression at the plasma membrane was increased. Antibody internalization experiments and subcellular fractionation analyses showed that immature MHC II were internalized from the plasma membrane but had limited access to lysosomes, explaining the reduced Ii chain degradation. Immunoelectron microscopy revealed that Nef expression induced a marked accumulation of multivesicular bodies (MVBs) containing Nef, MHC II, and high amounts of Ii chain. The Nef-induced up-regulation of surface Ii chain was inhibited by LY294002 exposure, indicating the involvement of a phosphatidylinositol 3-kinase, whose products play a key role in MVB biogenesis. Together, our results indicate that Nef induces an increase of the number of MVBs where MHC II complexes accumulate. Given that human immunodeficiency virus recruits the MVB machinery for its assembly process, our data raise the possibility that Nef is involved in viral assembly through its effect on MVBs.

Ludger Johannes, Valérie Pezo, Frédéric Mallard, Danièle Tenza, Aimée Wiltz, Agnès Saint-Pol, Julie Helft, Claude Antony, Philippe Benaroch (2003 Apr 26)

Effects of HIV-1 Nef on retrograde transport from the plasma membrane to the endoplasmic reticulum.

Traffic (Copenhagen, Denmark) : 323-32

Summary

HIV-1 Nef protein down-regulates several important immunoreceptors through interactions with components of the intracellular sorting machinery. Nef expression is also known to induce modifications of the endocytic pathway. Here, we analyzed the effects of Nef on retrograde transport, from the plasma membrane to the endoplasmic reticulum using Shiga toxin B-subunit (STxB). Nef expression inhibited access of STxB to the endoplasmic reticulum, but did not modify the surface expression level of STxB receptor, Gb3, nor its internalization rate as measured with a newly developed assay. Mutation of the myristoylation site or of a di-leucine motif of Nef involved in the interaction with the clathrin adaptor complexes AP1 and AP2 abolished the inhibition of retrograde transport. In contrast, mutations of Nef motifs known to interact with PACS-1, beta COP or a subunit of the v-ATPase did not modify the inhibitory activity of Nef on retrograde transport. Ultrastructural analysis revealed that Nef was present in clusters located on endosomal or Golgi membranes together with internalized STxB. Furthermore, in strongly Nef-expressing cells, STxB accumulated in endosomal structures that labeled with AP1. Our observations show that Nef perturbs retrograde transport between the early endosome and the endoplasmic reticulum. The potential transport steps targeted by Nef are discussed.

Year of publication 2002

Graça Raposo, Marilyn Moore, Donald Innes, Richtje Leijendekker, Andrew Leigh-Brown, Philippe Benaroch, Hans Geuze (2002 Sep 17)

Human macrophages accumulate HIV-1 particles in MHC II compartments.

Traffic (Copenhagen, Denmark) : 718-29

Summary

Macrophages are important targets for HIV-1 infection and harbor the virions in an as yet unidentified organelle. To determine the location of HIV-1 in these cells, an extensive analysis of primary human macrophages infected in vitro with HIV-1 was carried out by immuno-electron microscopy. Virus particles were found to accumulate in intracellular multivesicular compartments which were enriched in major histocompatibility complex class II molecules and CD63. These features are characteristics of major histocompatibility complex class II compartments where maturing class II molecules acquire their peptide cargo. The membrane-delimited, electron-dense virus particles of 100-110 nm diameter labeled strongly for HIV-1 p24 antigen, major histocompatibility complex class II molecules, CD63 and, to a lesser extent for HIV-1 gp120 envelope protein and Lamp 1. Our data suggest that virus particles may access the lumen of the major histocompatibility complex class II compartment by budding from the limiting membrane, thus acquiring proteins of this membrane such as class II and CD63. Viral assembly and budding would therefore occur in macrophages by a process similar to the formation of the internal vesicles in multivesicular bodies and at the same location. This could account for the particular content in lipids and proteins previously found in the membrane wrapping HIV particles. Our observations also suggest direct fusion of the virus containing major histocompatibility complex class II compartment with the plasma membrane, leading to massive release of viral particles into the extracellular medium.

Hélène Vincent-Schneider, Pamela Stumptner-Cuvelette, Danielle Lankar, Sabine Pain, Graça Raposo, Philippe Benaroch, Christian Bonnerot (2002 Jul 4)

Exosomes bearing HLA-DR1 molecules need dendritic cells to efficiently stimulate specific T cells.

International immunology : 713-22

Summary

Exosomes are small vesicles (60-100 nm) secreted by various cell types upon the fusion of endosomal compartments with the plasma membrane. Exosomes from antigen-presenting cells (APC), such as B lymphocytes and dendritic cells (DC), bear MHC class II molecules. In addition, the injection of DC-derived exosomes was reported to elicit potent T cell responses in vivo. Here, we analyzed the activation of specific T cells by MHC class II-bearing exosomes in vitro. The rat mast cell line, RBL-2H3, was engineered to express human class II molecules uniformly loaded with an antigenic peptide [HLA-DR1-hemagglutinin (HA)]. These cells secreted exosomes bearing DR1 class II molecules upon stimulation by a calcium ionophore or IgE receptor cross-linking. Exosomes bearing DR1-HA(306-318) complexes activated HA/DR1-specific T cells only weakly, whereas the cross-linking of such exosomes to latex

beads increased stimulation of specific T cells. By contrast, the incubation of free exosomes with DC resulted in the highly efficient stimulation of specific T cells. Thus, exosomes bearing MHC class II complexes must be taken up by professional APC for efficient T cell activation.

Pamela Stumptner-Cuvelette, Philippe Benaroch (2002 Feb 21)
Multiple roles of the invariant chain in MHC class II function.
Biochimica et biophysica acta : 1-13

Summary