Communicative & Integrative Biology 5:3, 291–293; May/June 2012; © 2012 Landes Bioscience

The unexpected role of Drosophila OCRL during cytokinesis

Khaled Ben El Kadhi,[1] Grégory Emery[2,3] and Sebastien Carreno[1,3,*]

[1]Cell Biology of Mitosis laboratory; Institute for Research and Immunology and Cancer; Montréal, Québec Canada; [2]Vesicular trafficking and Cell Signalling laboratory; Institute for Research and Immunology and Cancer; Montréal, Québec Canada; [3]Département de Pathologie et de Biologie Cellulaire; Université de Montréal; Montréal, Québec Canada

Inositides are intrinsic components of cell membranes that regulate a wide variety of cellular functions. PtdIns(4,5)P_2, one of the most abundant phosphoinositides, is restricted at the plasma membrane where it regulates numerous functions including cell division. We have recently established that the Drosophila inositol 5-phosphatase, dOCRL, is essential for cytokinesis, the last step of cell division (Ben El Kadhi et al. 2011).[8] We demonstrated that dOCRL is required for the dephosphorylation of PtdIns(4,5)P_2 at the surface of endosomes, resulting in the restriction of this phosphoinositide to the cell cortex during cytokinesis. dOCRL is the Drosophila ortholog of human OCRL1, a PtdIns(4,5)P_2 phosphatase mutated in the X-linked disorder oculocerebrorenal Lowe syndrome. Here, we discuss the relevance of our findings with reference to the role of human OCRL1 in non-pathological and pathological conditions.

Keywords: phosphoinositides, PtdIns(4,5)P2, cytokinesis, cell division, OCRL1, Lowe syndrome, intracellular trafficking

Submitted: 03/02/12

Accepted: 03/05/12

http://dx.doi.org/10.4161/cib.19914

*Correspondence to: Sebastien Carreno; Email: sebastien.carreno@umontreal.ca

Addendum to: Ben El Kadhi K, Roubinet C, Solinet S, Emery G, Carréno S. The inositol 5-phosphatase dOCRL controls PI(4,5)P_2 homeostasis and is necessary for cytokinesis. Curr Biol 2011; 21:1074–9; PMID:21658948; http://dx.doi.org/10.1016/j.cub.2011.05.030

Phosphoinositides are intrinsic components of cell membranes that regulate a wide variety of cellular functions.[1,2] The phosphoinositide family is composed of seven members that are synthesized through the phosphorylation of the 3-, 4- and/or 5-position of the inositol ring. Phosphoinositides are produced at cellular membranes and influence a number of processes including cell survival, vesicular trafficking, cytoskeletal reorganization, cell migration and cell division. Phosphoinositides bind and regulate a plethora of proteins at the interface of the cytosol and cellular membranes. The spatial compartmentalization of phosphoinositides is regulated by the action of specific kinases, phosphatases and phospholipases. This integrated signaling network controls phosphoinositide segregation on different cellular membranes and contributes to create specialized sub-membrane domains with specific biological functions.[3] For instance, during cell division, PtdIns(4,5)P_2 accumulates at the cleavage furrow where it plays essential roles during cytokinesis.[4-7]

We have recently reported an unexpected role of the Drosophila PtdIns(4,5)P_2 phosphatase OCRL (dOCRL) during cytkinesis.[8] Importantly, mutations in the OCRL1 gene, the human ortholog of dOCRL, are the cause of the Oculocerebrorenal syndrome of Lowe.[9] This rare X-linked genetic disease is characterized by mental retardation, congenital cataract and renal dysfunction. The OCRL1 gene encodes for two splice isoforms (OCRL1a and OCRL1b) that comprise a central inositol 5-phosphatase domain, a poorly characterized ASH domain and a catalytically inactive Rho-GAP domain.[10] In addition, OCRL1 isoforms were shown to bind with different affinity to clathrin. In vitro, the preferential substrate of OCRL1 is the phosphate at the 5- position of PtdIns(4,5)P_2.[11] Impairment of the inositol 5-phosphatase activity of OCRL1 is at the basis of the molecular dysfunctions causing the Lowe syndrome.[12] While the functions of OCRL1 have been subject to intense investigations, they still remain poorly understood. Difficulties in OCRL1 studies rely on the fact that cells also express INPP5B, an OCRL1 paralog that was shown to fulfill similar functions.[13] Drosophila expresses only one OCRL

ortholog, dOCRL, and we have recently demonstrated that its PtdIns(4,5)P_2 phosphatase activity was important to establish PtdIns(4,5)P_2 homeostasis, to regulate vesicular trafficking and to ensure the fidelity of cytokinesis.[8]

OCRL Proteins Regulate Homeostasis of PtdIns(4,5)P_2

Similarly to its human ortholog, dOCRL associates with the membrane of several class of endosomes.[8,14] dOCRL insures that PtdIns(4,5)P_2 pools are principally restricted at the plasma membrane by dephosphorylating this phosphoinositide on endomembranes. When dOCRL is knocked-down by RNAi, Drosophila cells in culture abnormally accumulate PtdIns(4,5)P_2 at the surface of giant endocytic vacuoles.[8] Interestingly, it has been recently reported that OCRL1 also regulates PtdIns(4,5)P_2 levels on endosomes of human cells. Similarly to what we observed in Drosophila, HeLa cells RNAi-depleted for OCRL1, present abnormal, enlarged endosomes enriched in PtdIns(4,5)P_2.[15] Therefore, regulation of PtdIns(4,5)P_2 homeostasis and control of endosomal morphology by OCRL proteins is a general mechanism conserved across evolution. In addition, the function of OCRL proteins in the establishment of PtdIns(4,5)P_2 homeostasis is likely to participate to the underlying causes of the Lowe syndrome since cells from patient suffering from this disease have been shown to present elevated levels of PtdIns(4,5)P_2.[16]

OCRL Proteins and Vesicular Trafficking

We established that dOCRL does not preferentially associate with one specific endosomal compartment, and is found at the surface of early, late and recycling endosomes. How dOCRL is targeted to these endosomes is not currently understood. However, two-hybrid experiments have revealed that its human ortholog, OCRL1, interacts with 16 members of the rab protein family, which regulate membrane trafficking.[17] Furthermore, it has been shown that rab5 and rab6 directly stimulate the inositol 5-phosphatase activity of OCRL1.[18] Therefore, it is tempting to speculate that rab proteins regulate OCRL proteins recruitment at the surface of endosomes to control homeostasis of PtdIns(4,5)P_2. Inactivation of OCRL proteins in Drosophila and in human, leads to a strong disorganization of the endocytic compartments with the apparition of enlarged endosomes.[8,15] In Drosophila, these large endocytic vacuoles appear to be the result of an unregulated fusion of early, late and recycling endosomes. The molecular explanation of this defect is still unclear but our observations suggest that the abnormal accumulation of PtdIns(4,5)P_2 on endocytic compartments, disrupt the phosphoinositide signature of each family of endosomes[19] and trigger homotypic fusion of these undefined endosomes.

OCRL Proteins and Cytokinesis

We found that when dOCRL is depleted by dsRNA, up to 40% of Drosophila cells fails cytokinesis and become multinucleated.[8] This cytokinesis failure is characterized by an abortive cleavage furrow that still forms but regresses rapidly and does not successively separate the two daughter cells. This phenotype is directly linked to the deregulation of PtdIns(4,5)P2 homeostasis: during cytokinesis, the abnormal accumulation of PtdIns(4,5)P2 on endomembrane mis-targets essential components of the cytokinetic ring such as rhoA, actin, myosin and anillin. These components are recruited on endomembranes, at the expense of the cleavage furrow, and cannot establish a stable, efficient, cortical cytokinetic ring. These observations brought into light the essential role of PtdIns(4,5)P2 as one of the major spatial cue that secures cleavage furrow positioning and stability during cytokinesis. The important role of dOCRL during cytokinesis appears to be conserved in human. RNAi depletion of OCRL1 in human cells delays abscission of the intercellular bridge connecting the two daughter cells after cytokinesis.[20] In human cells, rab35 recruit OCRL1 at the intercellular bridge where it locally dephosphorylates PtdIns(4,5)P2 and modifies lipid and actin composition. This remodeling of the intercellular bridge was proved to be necessary to the subsequent step of abscission. Accordingly, intercellular bridge of cells from Lowe syndrome patient show an abnormal accumulation of PtdIns(4,5)P2 and actin that delays cell abscission. While OCRL proteins are important for cytokinesis in Drosophila and in mammals, their inactivation does not trigger similar cell division defects. One possible explanation is that human cells also express INPP5B that could partially substitute for OCRL1 function during cell division. Interestingly, we have found that human INPP5B could partially rescue dOCRL function during cytokinesis in Drosophila cells (unpublished results). These two studies have identified a novel and unexpected role of OCRL proteins during cell division.[8,20] Drosophila which expresses only one ortholog of OCRL1 appears as a powerful and promising model to further dissect in vivo the different functions of proteins of the OCRL family.

Acknowledgments

K.B.E.K. is a recipient of IRIC, UdeM and Canadian Institutes for Health Research scholarships. G.E. is recipient of a Tier II Canada Research Chair in Vesicular Transport and Cell Signaling. S.C. is the recipient of a Fonds de la Recherche en Santé du Québec Junior 1. This work was supported by grants from the Canadian Institutes for Health Research (S.C.: MOP-89877, G.E.: MOP-114899) and from the Cancer Research Society (G.E.).

References

1. Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. Nature 2006; 443:651-7; PMID:17035995; http://dx.doi.org/10.1038/nature05185
2. Vicinanza M, D'Angelo G, Di Campli A, De Matteis MA. Function and dysfunction of the PI system in membrane trafficking. EMBO J 2008; 27:2457-70; PMID:18784754; http://dx.doi.org/10.1038/emboj.2008.169
3. Kutateladze TG. Translation of the phosphoinositide code by PI effectors. Nat Chem Biol 2010; 6:507-13; PMID:20559318; http://dx.doi.org/10.1038/nchembio.390
4. Emoto K, Inadome H, Kanaho Y, Narumiya S, Umeda M. Local change in phospholipid composition at the cleavage furrow is essential for completion of cytokinesis. J Biol Chem 2005; 280:37901-7; PMID:16162509; http://dx.doi.org/10.1074/jbc.M504282200
5. Field SJ, Madson N, Kerr ML, Galbraith KA, Kennedy CE, Tahiliani M, et al. PtdIns(4,5)P2 functions at the cleavage furrow during cytokinesis. Curr Biol 2005; 15:1407-12; PMID:16085494; http://dx.doi.org/10.1016/j.cub.2005.06.059
6. Roubinet C, Decelle B, Chicanne G, Dorn JF, Payrastre B, Payre F, et al. Molecular networks linked by Moesin drive remodeling of the cell cortex during mitosis. J Cell Biol 2011; 195:99-112; PMID:21969469; http://dx.doi.org/10.1083/jcb.201106048
7. Wong R, Hadjiyanni I, Wei HC, Polevoy G, McBride R, Sem KP, et al. PIP2 hydrolysis and calcium release are required for cytokinesis in Drosophila spermatocytes. Curr Biol 2005; 15:1401-6; PMID:16085493; http://dx.doi.org/10.1016/j.cub.2005.06.060
8. Ben El Kadhi K, Roubinet C, Solinet S, Emery G, Carréno S. The inositol 5-phosphatase dOCRL controls PI(4,5)P_2 homeostasis and is necessary for cytokinesis. Curr Biol 2011; 21:1074-9; PMID:21658948; http://dx.doi.org/10.1016/j.cub.2011.05.030
9. Lowe M. Structure and function of the Lowe syndrome protein OCRL1. Traffic 2005; 6:711-9; PMID:16101675; http://dx.doi.org/10.1111/j.1600-0854.2005.00311.x
10. Choudhury R, Noakes CJ, McKenzie E, Kox C, Lowe M. Differential clathrin binding and subcellular localization of OCRL1 splice isoforms. J Biol Chem 2009; 284:9965-73; PMID:19211563; http://dx.doi.org/10.1074/jbc.M807442200
11. Zhang X, Jefferson AB, Auethavekiat V, Majerus PW. The protein deficient in Lowe syndrome is a phosphatidylinositol-4,5-bisphosphate 5-phosphatase. Proc Natl Acad Sci U S A 1995; 92:4853-6; PMID:7761412; http://dx.doi.org/10.1073/pnas.92.11.4853
12. Leahey AM, Charnas LR, Nussbaum RL. Nonsense mutations in the OCRL-1 gene in patients with the oculocerebrorenal syndrome of Lowe. Hum Mol Genet 1993; 2:461-3; PMID:8504307; http://dx.doi.org/10.1093/hmg/2.4.461
13. Jänne PA, Suchy SF, Bernard D, MacDonald M, Crawley J, Grinberg A, et al. Functional overlap between murine Inpp5b and Ocrl1 may explain why deficiency of the murine ortholog for OCRL1 does not cause Lowe syndrome in mice. J Clin Invest 1998; 101:2042-53; PMID:9593760; http://dx.doi.org/10.1172/JCI2414
14. Ungewickell A, Ward ME, Ungewickell E, Majerus PW. The inositol polyphosphate 5-phosphatase Ocrl associates with endosomes that are partially coated with clathrin. Proc Natl Acad Sci U S A 2004; 101:13501-6; PMID:15353600; http://dx.doi.org/10.1073/pnas.0405664101
15. Vicinanza M, Di Campli A, Polishchuk E, Santoro M, Di Tullio G, Godi A, et al. OCRL controls trafficking through early endosomes via PtdIns4,5P_2-dependent regulation of endosomal actin. EMBO J 2011; 30:4970-85; PMID:21971085; http://dx.doi.org/10.1038/emboj.2011.354
16. Zhang X, Hartz PA, Philip E, Racusen LC, Majerus PW. Cell lines from kidney proximal tubules of a patient with Lowe syndrome lack OCRL inositol polyphosphate 5-phosphatase and accumulate phosphatidylinositol 4,5-bisphosphate. J Biol Chem 1998; 273:1574-82; PMID:9430698; http://dx.doi.org/10.1074/jbc.273.3.1574
17. Fukuda M, Kanno E, Ishibashi K, Itoh T. Large scale screening for novel rab effectors reveals unexpected broad Rab binding specificity. Mol Cell Proteomics 2008; 7:1031-42; PMID:18256213; http://dx.doi.org/10.1074/mcp.M700569-MCP200
18. Hyvola N, Diao A, McKenzie E, Skippen A, Cockcroft S, Lowe M. Membrane targeting and activation of the Lowe syndrome protein OCRL1 by rab GTPases. EMBO J 2006; 25:3750-61; PMID:16902405; http://dx.doi.org/10.1038/sj.emboj.7601274
19. Behnia R, Munro S. Organelle identity and the signposts for membrane traffic. Nature 2005; 438:597-604; PMID:16319879; http://dx.doi.org/10.1038/nature04397
20. Dambournet D, Machicoane M, Chesneau L, Sachse M, Rocancourt M, El Marjou A, et al. Rab35 GTPase and OCRL phosphatase remodel lipids and F-actin for successful cytokinesis. Nat Cell Biol 2011; 13:981-8; PMID:21706022; http://dx.doi.org/10.1038/ncb2279