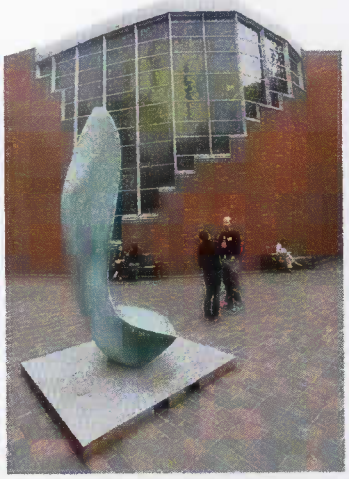
BIOCHEMICAL SOCIETY

FEBS

Programme & Abstracts

A joint Biochemical Society/FEBS Focused Meeting

Signalling 2015: Cellular Functions of Phosphoinositides and Inositol Phosphates



1–4 September 2015
Robinson College, Cambridge, UK

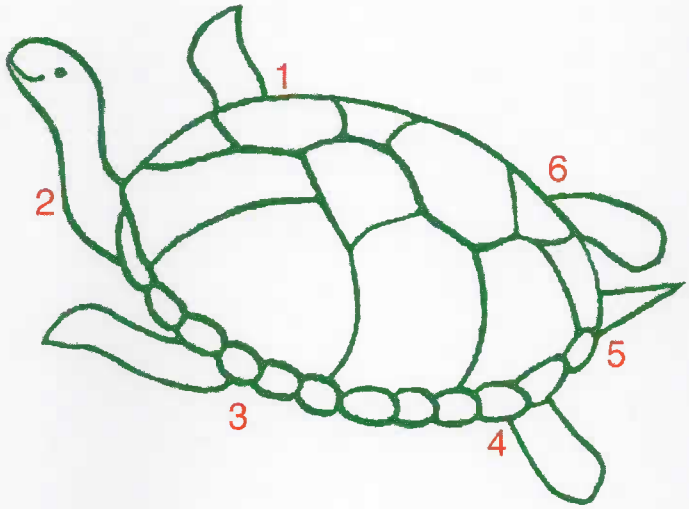


Image kindly supplied by Phillip Hawkins (Babraham Institute, UK)

www.biochemistry.org

Sponsored by:

addgene
A better way to share plasmids

BMG LABTECH

EMBO

AstraZeneca

Babraham Institute

greiner bio-one

Avanti® Polar Lipids, Inc.

Cell Signaling Technology

RespiVert
Discovering inhaled medicines

Typeset by Portland Press Limited and printed in Great Britain by Metro Commercial Printing Ltd, Watford

P035 Endosomal remodelling during early phase of murine cytomegalovirus infection

Ljerka Karleuša[1], Kristina Grabušić[2], Hana Mahmutefendić[1], Gordana Blagojević Zagorac[1], Maja Ilić Tomaš[3] and Pero Lučin[1]
[1]Department of Physiology and Immunology, School of Medicine, University of Rijeka, Rijeka, Croatia
[2]Department of Biotechnology, University of Rijeka, Rijeka, Croatia
[3]Clinical Hospital Centre Rijeka, Rijeka, Croatia

Mouse cytomegalovirus (MCMV) is a large DNA virus with a number of genes that manipulate cellular functions. Among other alterations, MCMV reorganizes host's cell endosomal system in order to establish environment for virion envelopment. That process starts early in the infection and continues throughout the entire replication cycle until the assembly compartment is established at the beginning of the late phase of infection. Early events of endosomal reorganization were in the focus of our study.
We infected Balb3T3 fibroblasts with ΔMC95.15 – MCMV with deleted m138 gene that encodes a protein with immunoglobulin binding capacity (viral FcR). Selected markers of endocytic pathways were followed by immunofluorescence and confocal microscopy. The intracellular routes were determined by functional assays. Western-blot analysis was used to elucidate intracellular expression of endosome regulating proteins (Rab and Arf family).
The endosomal remodeling was apparent at 6 hrs post infection as juxtanuclear vacuole-tubular compartment(s) that retained early endosomal cargo molecules (TfR, MHC-I) and markers (EEA1, Rab5). This compartment was characterized using a set of early endosomal as well as "early" (MLN64) and "late" (NPC1) late endosomal markers. The endosomal system reshaping was associated with rapid down-regulation of Rab proteins that regulate endosomal recycling (Rab4, Rab22a, Rab11, Arf1), early-to-late endosome transit (Rab7, Rab9) or late endosomal recycling (Rab27a). This suggests that MCMV uses a mechanism of rapid Rab or Arf protein degradation in order to reshape endosomal system.

Notes:

P036 Conditional inactivation of the class II PI3K-C2α in mice

York Posor, Maria Whitehead and Bart Vanhaesebroeck
University College London, London, UK

The class I PI3Ks have been intensely investigated because of their roles as signal transducers in metabolism, immunity, and cancer. The physiological functions of the class II isoforms, however, remain poorly understood.
We have previously uncovered a role for the class II PI3K-C2α in regulating clathrin-mediated endocytosis, and this isoform has further been implicated in exocytosis and endocytic recycling. At the physiological level, embryonic lethality of homozygous knock-outs has been linked to defective angiogenesis and hedgehog signalling. However, roles of PI3K-C2α in post natal stages have not been addressed and selective inhibitors of this isoform are not available at present.
We have therefore generated a conditional mouse model for the inactivation of PI3K-C2α *in vivo*. In contrast to the KO, this model allows specifically addressing kinase-dependent functions and hence is a superior model to evaluate PI3K-C2α as a potential drug target. Analysis of primary embryonic fibroblasts isolated from these mice confirmed success of the gene targeting strategy and showed near complete inactivation at five days after inducing Cre-recombinase. Additionally, *in vivo* inactivation by oral administration of tamoxifen has been demonstrated and is currently being optimized in order to maximize recombination efficiency across tissues. We will use this model to uncover the physiologically relevant roles of PI3K-C2α and evaluate its potential as a drug target.

Notes: