Autophagy

Autophagy

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/kaup20

PRKA/PKA signals and autophagy: space matters

Liliana Felicia Iannucci, Giulietta Di Benedetto & Konstantinos Lefkimmiatis

To cite this article: Liliana Felicia Iannucci, Giulietta Di Benedetto & Konstantinos Lefkimmiatis (2021) PRKA/PKA signals and autophagy: space matters, Autophagy, 17:6, 1563-1564, DOI: 10.1080/15548627.2021.1924501

To link to this article: https://doi.org/10.1080/15548627.2021.1924501

Published online: 10 May 2021.

AUTOPHAGY
2021, VOL. 17, NO. 6, 1563–1564
https://doi.org/10.1080/15548627.2021.1924501

Taylor & Francis
Taylor & Francis Group

AUTOPHAGIC PUNCTUM

PRKA/PKA signals and autophagy: space matters

Liliana Felicia Iannucci[a,b], Giulietta Di Benedetto[b,c], and Konstantinos Lefkimmiatis [a,b]

[a]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [b]Foundation for Advanced Biomedical Research, Veneto Institute of Molecular Medicine, Padua, Italy; [c]Neuroscience Institute, National Research Council, Padua Section, Padua, Italy

ABSTRACT

Macroautophagy/autophagy is the cellular process responsible for the elimination and recycling of aggregated proteins and damaged organelles. Whereas autophagy is strictly regulated by several signaling cascades, the link between this process and the subcellular distribution of its regulatory pathways remains to be established. Our recent work suggests that the compartmentalization of PRKA/PKA (protein kinase cAMP-activated) determines its effects on autophagy. We found that increased cAMP levels generate dramatically different PRKA activity "signatures" mainly dependent on the actions of phosphatases and the distribution of the PRKA holoenzymes containing type II regulatory subunits (PRKAR2A and PRKAR2B; RII). In this punctum we discuss how compartmentalized PRKA signaling events are generated and affect the autophagic flux in specific cell types.

ARTICLE HISTORY
Received 17 April 2021
Revised 27 April 2021
Accepted 27 April 2021

KEYWORDS
PRKA; PKA; autophagy; cAMP; phosphatases; compartmentalization

The autophagic process is a multistep highly regulated mechanism important for maintaining cellular homeostasis. It occurs in a time- and space-controlled manner and eliminates potentially harmful, damaged or unnecessary cellular components. Post-translational modifications of the autophagic machinery make it possible to finely regulate this process in each of its steps. Alongside the serine/threonine kinase MTOR, which is the master regulator of autophagy, other kinases are involved, including PRKA/PKA (protein kinase cAMP-activated).

In our recent study [1] we used different fluorescent reporters to monitor autophagy and found that induction of the cAMP-PRKA axis affects autophagy in HT-29 cells, a human colon adenocarcinoma cell line; however, similar cAMP increases do not affect this process in HeLa cells. These unexpected findings were confirmed also by monitoring the endogenous autophagic marker MAP1LC3/LC3 (microtubule associated protein 1 light chain 3) and raised several questions about the molecular mechanism(s) underlying these differences.

To solve some of these queries, we used two types of FRET-based sensors to monitor the cellular levels of free cAMP (a RAPGEF-based construct, EPAC-S^{H187}) and PRKA-dependent phosphorylation (AKAR4). cAMP measurements confirmed that both HT-29 and HeLa cells produce similar levels of cAMP, especially in the presence of the PDE (phosphodiesterase) inhibitor isobutyl-methylxanthine(IBMX), to block cAMP degradation. Surprisingly, despite virtually identical levels of PRKA enzyme and cAMP, the two cell lines display dramatically different PRKA-dependent phosphorylation. In HeLa cells PRKA-dependent phosphorylation mirrors the increases of cAMP, while, on the contrary, in HT-29 cells extremely low PRKA activity is measured, even at saturating levels of the messenger. In line with these findings, the PRKA-dependent phosphorylation patterns of endogenous targets in the two models are different; however, increased cAMP synthesis results in the phosphorylation of a number of endogenous proteins also in HT-29 cells.

Does the connection between autophagy and PRKA rely on PRKA-induced phosphorylation levels or localization? Our experiments suggest that the latter is most likely the case. In fact, upon cAMP increases we detect high levels of diffuse PRKA-dependent phosphorylation in HeLa cells (without any accompanying effect on autophagy), whereas, conversely, autophagy is clearly affected in HT-29 cells, that display rather modest phosphorylation of soluble targets. The difference between HeLa and HT-29 cells is the subcellular distribution of PRKA phosphorylated targets. In fact, in HT-29 cells, contrary to HeLa, PRKA-dependent phosphorylation is clearly detectable at the endoplasmic reticulum and mitochondria, independently from cAMP levels. This was surprising because the compartmentalization of these events persisted even after PDE inhibition, which is expected to abolish cAMP microdomains. Nevertheless, these data are in line with our previous finding that phosphatases offer a novel mechanism for shaping in space and time PRKA-driven phosphorylation events, independently of cAMP. This was proven to be the mechanism regulating PRKA signals in HT-29 cells; in fact by simply inhibiting a subset of phosphatases (PPP2/PP2A) the signaling differences between these cells and HeLa are abolished.

To directly connect compartmentalized PRKA moieties to the effects on autophagy observed in HT-29 cells, we employed two genetically encoded disruptor peptides able to selectively displace from their subcellular sites the PRKA holoenzymes containing regulatory subunits PRKAR1A and PRKAR1B (RI) or RII. Tethering of PRKA to AKAPs (A-kinase anchoring proteins) depends on the PRKA regulatory subunits and determines the subcellular localization of the enzyme. Interestingly, displacement of RII-based, but not RI-based, PRKA reverts the increases in autophagic flux induced by cAMP, providing

CONTACT Konstantinos Lefkimmiatis konstantinos.lefkimmiatis@unipv.it Department of Molecular Medicine, University of Pavia, Via Carlo Forlanini 6, 27100 Pavia (PV), Italy

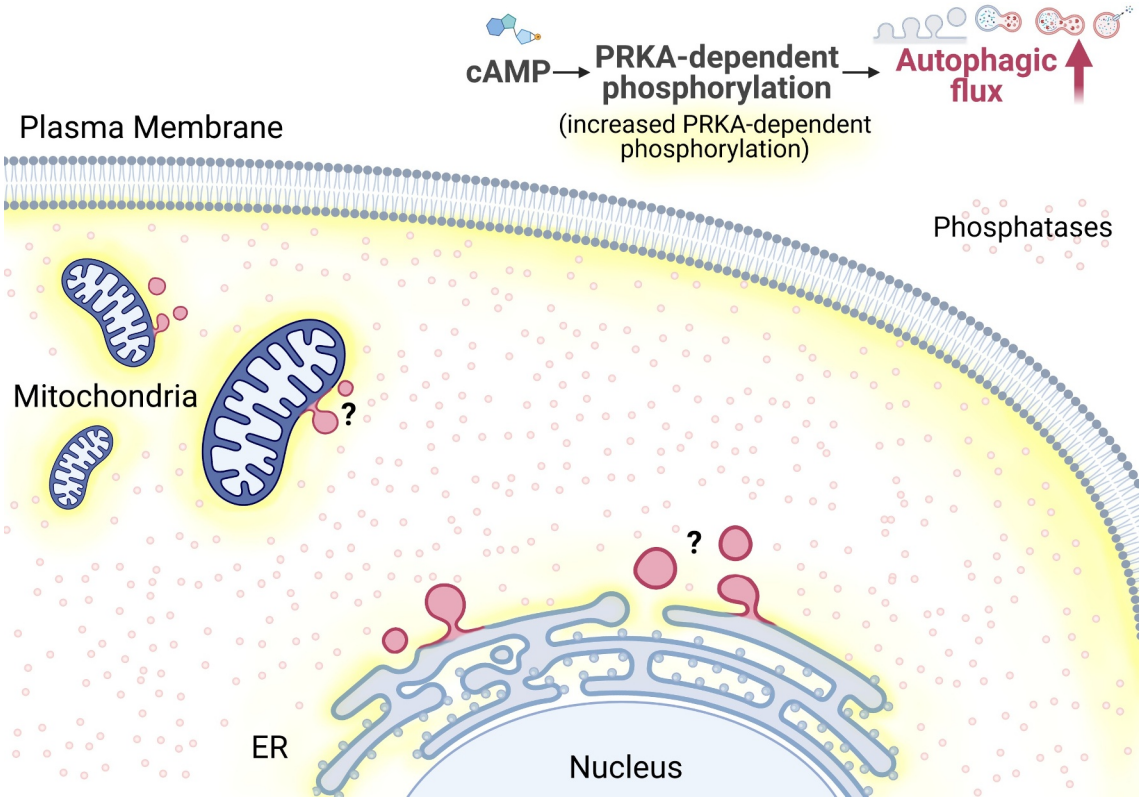

Figure 1. PRKA-dependent phosphorylation is curbed in the cytosol by soluble phosphatases, which are less efficient in affecting PRKA targets that are phosphorylated near membranes. The local increase of PRKA target phosphorylation facilitates the autophagic process. The PRKA microdomain(s) directly involved in the regulation of autophagy remains to be established. ER: endoplasmic reticulum. Image created with BioRender.com.

concrete evidence of a link between autophagy and compartmentalized PRKA.

There are a number of unsolved questions regarding the post-translational control of autophagy, and our work adds a new level of complexity and raises its own set of new questions. Which are the PRKA moieties involved in the control of autophagy? From our experiments, we could exclude PRKA located at the vicinity of the plasma membrane. In fact, these moieties behave in a similar manner also in HeLa cells (where autophagy is insensitive to cAMP increases). It is difficult to define an exclusive role between the endoplasmic reticulum and mitochondria, both valid candidates with similar PRKA content and activity at their surface. It will also be interesting to better understand at which stage of autophagy compartmentalized PRKA events are involved. Our experiments using chloroquine, to inhibit the formation of autolysosomes, suggest that RII-based PRKA activation participates in the stages before this step; however, the exact point between the recognition of the cargo, the formation of the phagophore membrane and the maturation of the autophagosome remains to be determined.

Another important question that is raised by our study is the identity of the PRKA targets that participate in the autophagic control. It is reasonable to expect that localized PRKA will phosphorylate its already known autophagic targets such as LC3, RPTOR/RAPTOR (regulatory associated protein of MTOR complex 1) and several ATG (autophagy related) proteins. However, it is also tempting to speculate that other targets could be involved in this newly discovered regulatory pathway.

Our experiments showed that the localized activity of RII-based PRKA holoenzymes affects the autophagic flux, and connect for the first time the subcellular localization of a regulator to the autophagic process (Figure 1). In the light of these findings, it becomes important to consider how the spatiotemporal compartmentalization of the signaling pathways that regulate autophagy may be connected to the pathophysiology of autophagy-related disease.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was funded by grants from the Foundation "Cassa di Risparmio di Padova e Rovigo" [CA.RI.PA.RO] and the Italian Ministry of University and Scientific Research Scientific Research [PRIN-2017BF3PXZ to KL].

ORCID

Konstantinos Lefkimmiatis http://orcid.org/0000-0001-7137-7866

Reference4

[1] Grisan F, Iannucci LF, Surdo NC, et al. PKA compartmentalization links cAMP signaling and autophagy. Cell Death Differ. 2021. DOI:10.1038/s41418-021-00761-8