


DRAMing for autophagy

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Autophagy, a catabolic lysosomal recycling pathway, is often found dysregulated in human diseases. Whereas its prime cell stress-related function is cytoprotection, autophagy has also been linked to the activation of apoptosis and cell death. One group of proteins which participates in the orchestration of autophagy and apoptosis is the family of DRAM proteins. In the current issue of *The FEBS Journal*, Barthelet et al. uncover a compensatory crosstalk between the two newest members of the family, DRAM-4 and DRAM-5, the latter one regulating autophagic activity.

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Introduction

Multiple stress-response pathways support eukaryotic cell survival under adverse environmental conditions. One of these pathways, triggered by a wide variety of stressors, is macroautophagy (hereafter referred to as autophagy), an evolutionarily conserved lysosomal degradation pathway. Upon initiation, a new isolation membrane forms, which encapsulates cytoplasmic components targeted for degradation, including selected proteins, entire organelles or invading pathogens. The closing of the membrane results in the formation of a double membrane-vesicle called autophagosome, which matures and eventually fuses with lysosomes forming an autolysosome [1]. After fusion, lysosomal hydrolases degrade the autophagic cargo, as well as the inner membrane. Breakdown products such as amino acids and fatty acids are transported back into the cytosol, where they are reused to fuel catabolic or vital anabolic reactions. In general, autophagy is associated with cell survival under stress conditions; however, it has also been shown to support cell death and to act as a pro-apoptotic factor in a context-dependent manner.

Abbreviations

DRAM, DRAM1-related/associated members.

The DRAM proteins

With the discovery and characterisation of the DRAM1-Related/Associated Members (DRAM) protein family, Kevin Ryan's research group uncovered new molecular links between autophagy and apoptosis regulation. The founding family member DRAM-1 was identified as a direct p53 target gene [2], its expression being also modulated by JNK [3]. DRAM-1 is a transmembrane protein located on lysosomes and other components along the endocytic pathway. DRAM-1 is necessary for p53-induced autophagy and also acts as a pro-apoptotic factor. Coherent with its pro-apoptotic effect, it was shown that DRAM-1 is commonly less abundant in primary tumours compared to healthy tissue. Following this discovery, four homologues – DRAM-2 to 5 – with varying degrees of sequence identity and conservation were found in humans [4], all of them being transmembrane proteins and localising to the plasma membrane and/or the endo-lysosomal compartment [4,5]. DRAM orthologues were also identified and characterised in drosophila and zebrafish [6],

establishing DRAM-1 as an evolutionarily conserved regulator of autophagy [4].

So far, the most studied member of the family remains DRAM-1. Similar to autophagy itself, the role of DRAM-1 in cancer also seems to be context-dependent. Although DRAM-1 was characterised as pro-apoptotic and having tumour suppressive functions in squamous cell carcinomas [2], it was also linked to poor outcome in different cancer types. Glioblastoma multiforme stem cells express high levels of DRAM-1, which in this context promotes metabolic reprogramming and enhances cell migration and invasiveness [7]. p73, a regulator of apoptosis closely related to p53, was also shown to induce DRAM-1 expression. Interestingly, p73-induced DRAM-1 expression seems to not affect autophagy [8], but is important for neutrophil differentiation of acute promyelocytic leukaemia cells [9].

DRAM-1 was also shown to play a role in the case of infection and xenophagy, that is, the selective removal of pathogens by autophagy. Following mycobacterial infection, it is expressed under MYD88 control and colocalises with intracellular mycobacteria, promoting their autophagy-dependent lysosomal removal [6]. Interestingly, upon HIV infection, DRAM-1 expression is regulated by p53, promoting lysosomal membrane permeabilisation and leading to autophagy-independent apoptosis [10]. Thus, whereas DRAM-1 plays a clear role in autophagy regulation, its consequences on cell fate seem highly context-dependent. This might be partially due to the fact that the effects of DRAM-1 on lysosome biology seem to be broader than the regulation of autophagy. DRAM-1 was shown to contribute to lysosome acidification [11] and mTORC1 activation [12]. In the event of amino acid scarcity, DRAM-1 redirects newly synthesised amino acid transporters from their way to the plasma membrane to the lysosome, where they regulate amino acid transport from the lysosome to the cytosol, ultimately leading to mTORC1 reactivation [12].

The functions of DRAM-2, the closest homologue of DRAM-1 which is not induced by p53 and p73, in autophagy, as well as cell death, also seem multifaceted and context-dependent. DRAM-2 was shown to regulate autophagy in some cases, but not in others [4,13]. In ovarian cancer, it was shown to play a synergistic role with DRAM-1 at the lysosomal membrane, promoting apoptosis of tumour cells [14]. However in non-small cell lung cancer, DRAM-2 was shown to have oncogenic potential by promoting invasiveness and repressing p53 expression [15]. Interestingly, the latter study reported a diffuse cytoplasmic staining pattern of DRAM-2, indicating that different subcellular localisations may encode different functions. The

primary characterisation of DRAM-3, which is also not induced by p53, revealed its cellular localisation at the plasma membrane, endosomes and (auto)lysosomes. It was shown to induce autophagy flux and to promote cell survival in the absence of glucose, the latter effect being independent of its role in the regulation of autophagy [16].

DRAM-4 and DRAM-5

In the current issue of *The FEBS Journal*, Kevin Ryan, Valentin Barthet and colleagues provide the first detailed autophagy-relevant characterisation of the last two members of the DRAM family, DRAM-4 and DRAM-5 [17]. Fluorescent microscopy localises DRAM-4 and DRAM-5 mainly at endosomes and the plasma membrane respectively. Similar to DRAM-2 and DRAM-3, DRAM-4 and DRAM-5 expression is not regulated by p53 and in accordance, DNA damaging agents do not cause an increase in their expression. In contrast, expression of DRAM-4 and DRAM-5 is induced by nutrient deprivation in a stimuli- and cell line-dependent manner. Overexpression of both DRAM-4 and DRAM-5 leads to an increase in LC3B-II levels. Interestingly, in the case of DRAM-5, this is due to an increase in autophagosome biogenesis, that is, an increased autophagy flux. In the case of DRAM-4, this is due to a reduced autophagosome turnover, that is, a reduced autophagy flux. Complementing these overexpression studies, CRISPR/Cas-9-based knockouts of the endogenous genes indicate that *DRAM5* deletion does not result in any significant changes in LC3B-II accumulation; however, *DRAM4* deletion leads to a DRAM-5-dependent increase in autophagy flux. In agreement with this finding and further confirming the compensatory upregulation of DRAM-5 in the absence of DRAM-4, the authors continue to show that (i) autophagy is not induced in *DRAM4/DRAM5* double knockout cells and (ii) the deletion of *DRAM4* increases cell survival in the absence of glucose, serum or amino acids in a DRAM-5-dependent manner, presumably via the upregulation of autophagy (Fig. 1).

Conclusion

Autophagy and its dysregulations have been linked to numerous diseases. With their work, Kevin Ryan, Valentin Barthet and coworkers uncovered new DRAM family members as modulators of autophagy and demonstrated their crosstalk and roles under different stress conditions [17]. Similar to DRAM-1, the regulation of which is complex and context-dependent, further characterisation of DRAM-4 and DRAM-5

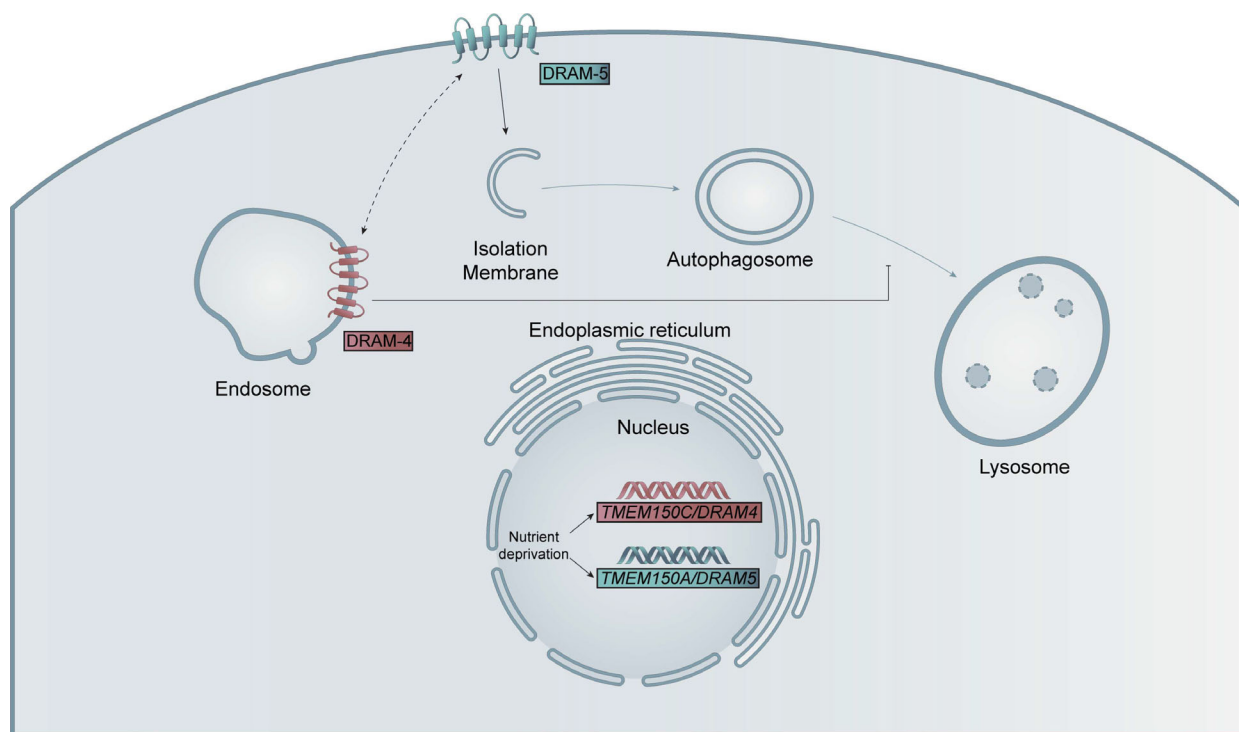


Fig. 1. DRAM-4 and DRAM-5 are both induced by nutrient starvation and reciprocally modulate autophagy.

will likely reveal new context-dependent regulations and modes of action. Although both proteins were mainly found at the endosome and plasma membrane in the current study, a context-dependent lysosomal localisation cannot be ruled out. This would open the door to more effects on lysosome biology beyond the regulation of autophagy, especially in the context of lysosomal membranes being important platforms regulating metabolic signalling. Characterisation of stimulus-dependent modifications and protein–protein interactions, for example, by proteomic approaches [1], would be a next step to help further understanding molecular events in DRAM-4 and DRAM-5 biology.

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Conflict of interest

The authors declare no conflict of interest.

Authors contributions

Writing, review, & editing: AL and JD.

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