

# KRS: A cut away from release in exosomes

Catherine Rabouille<sup>1,2</sup>

<sup>1</sup>Hubrecht Institute of the Royal Netherlands Academy of Arts and Sciences/University Medical Center Utrecht, Utrecht, Netherlands

<sup>2</sup>Department of Cell Biology, University Medical Center Groningen, Groningen, Netherlands

Cancer cells often trigger an inflammatory process, which in some cases may be driven by the presence of lysyl-tRNA synthetase (KRS) in the medium. Kim et al. (2017). *J. Cell Biol.* <https://doi.org/10.1083/jcb.201605118> now demonstrate that cleavage of the KRS by caspase-8 inside cells triggers its interaction with syntenin and its release in inflammatory exosomes.

Aminoacyl-tRNA synthetases (ARSs) are intriguing enzymes. Intracellularly, they catalyze the covalent attachment of amino acids to tRNAs and are key regulators of protein translation. However, these housekeeping enzymes have many other tricks up their sleeves. In the cell, several ARSs are also able to regulate gene expression at the level of transcription, splicing, and translation but via noncatalytic and unique mechanisms. ARSs also have different functions in the extracellular space, where they can elicit cytokine signaling responses that control angiogenesis, induce immune and proinflammatory gene expression programs, and trigger cell migration or apoptosis. The response elicited by particular ARSs is specific to the target cells (Son et al., 2014). For instance, when present in the extracellular medium, lysyl-tRNA synthetase (KRS) binds to macrophages and monocytes and activates MAPK signaling pathways that induce macrophage migration and TNF production (Park et al., 2005). How ARSs are released to the extracellular medium to carry out these activities is unknown. ARSs do not contain a signal peptide, and pharmacological agents blocking secretion through the secretory pathway have no effect on the amount of ARS in the medium. For a while, the presence of ARSs in the extracellular medium was thus thought to be caused by their passive release from cells that have undergone necrosis. In this issue, Kim et al. reinvestigate how KRS is released from cancer cells and find that this occurs through a caspase-8- and syntenin-dependent incorporation of KRS in exosomes.

Proteins that include a signal peptide are secreted through the conventional secretory pathway to the extracellular medium, but leaderless proteins (i.e., proteins without a signal peptide) are also found in the medium. It is now clear that these leaderless proteins can be unconventionally delivered via at least three distinct mechanisms that do not involve cell lysis. The first mechanism is translocation across the plasma membrane (the so-called type I unconventional protein secretion [UPS]) either through self-pores and auto-transportation (as seen with FGF2 and HIV-TAT [trans-activator of transcription]) or by the use of a transporter whose nature, in most cases, remains to be defined. The second mechanism is the type III UPS in which the lumen

of a membranous intermediate (be it an autophagosome, a late endosome, or the compartment for unconventional protein secretion [CUPS]) becomes enriched in leaderless cargo. These compartments then fuse with the plasma membrane to release their contents to the extracellular medium. Note that in both of these types of unconventional secretion, the released cargo is not meant to be membrane encapsulated (Rabouille, 2017). Third, leaderless proteins can also be released in the medium via extracellular vesicles that have many origins, such as plasma membrane blebs, apoptotic bodies, and exosomes.

Exosomes were discovered in the mid-1990s when the release of intraluminal vesicles from multivesicular bodies in B lymphocytes was observed (Colombo et al., 2014). Exosomes have been shown to be released from many cell types in vitro and vivo, and they mediate the spreading of different types of molecules from cells to cells (Colombo et al., 2014). Exosome formation starts with the invagination of the limiting membrane of an endosome in a manner that is dependent on the multimeric endosomal sorting complexes required for transport (ESC RT) complex (Babst, 2011) and ALIX (Bissig and Gruenberg, 2014). The syntenin–syndecan complex that is present at the surface of endosomes also appears to play an important role in exosome formation (Baietti et al., 2012). Exosomes are characterized by their homogenous size (~100 nm), their cup shape, and their purification characteristics on flotation gradients. They contain membrane proteins as well as cytosolic leaderless proteins and RNAs that are incorporated during the process of vesicle formation. Whether the cargo is specifically incorporated and concentrated in internal vesicles and how this is controlled remains to be investigated in most cases. Endosomes containing these internal vesicles, the so-called multivesicular bodies, fuse with the plasma membrane and release the vesicles into the extracellular medium as exosomes (Fig. 1; Colombo et al., 2014). To distinguish this third mechanism of unconventional secretion that yields cargo encapsulated in a membrane from the other two aforementioned mechanisms, I will call it “exosome release” and not secretion.

KRS can bind to syntenin, a key factor for the formation of exosomes. This led Kim et al. (2017) to investigate whether the exosome release pathway accounts for the presence of KRS in the medium. First, they found that KRS and syntenin are recovered in the same flotation gradient fraction that is consistent with localization in exosomes. Second, KRS release was strongly inhibited upon Rab35 and Rab27 depletion, two Rabs involved in the process of exosome formation and release (Hsu

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Correspondence to Catherine Rabouille: [c.rabouille@hubrecht.eu](mailto:c.rabouille@hubrecht.eu)



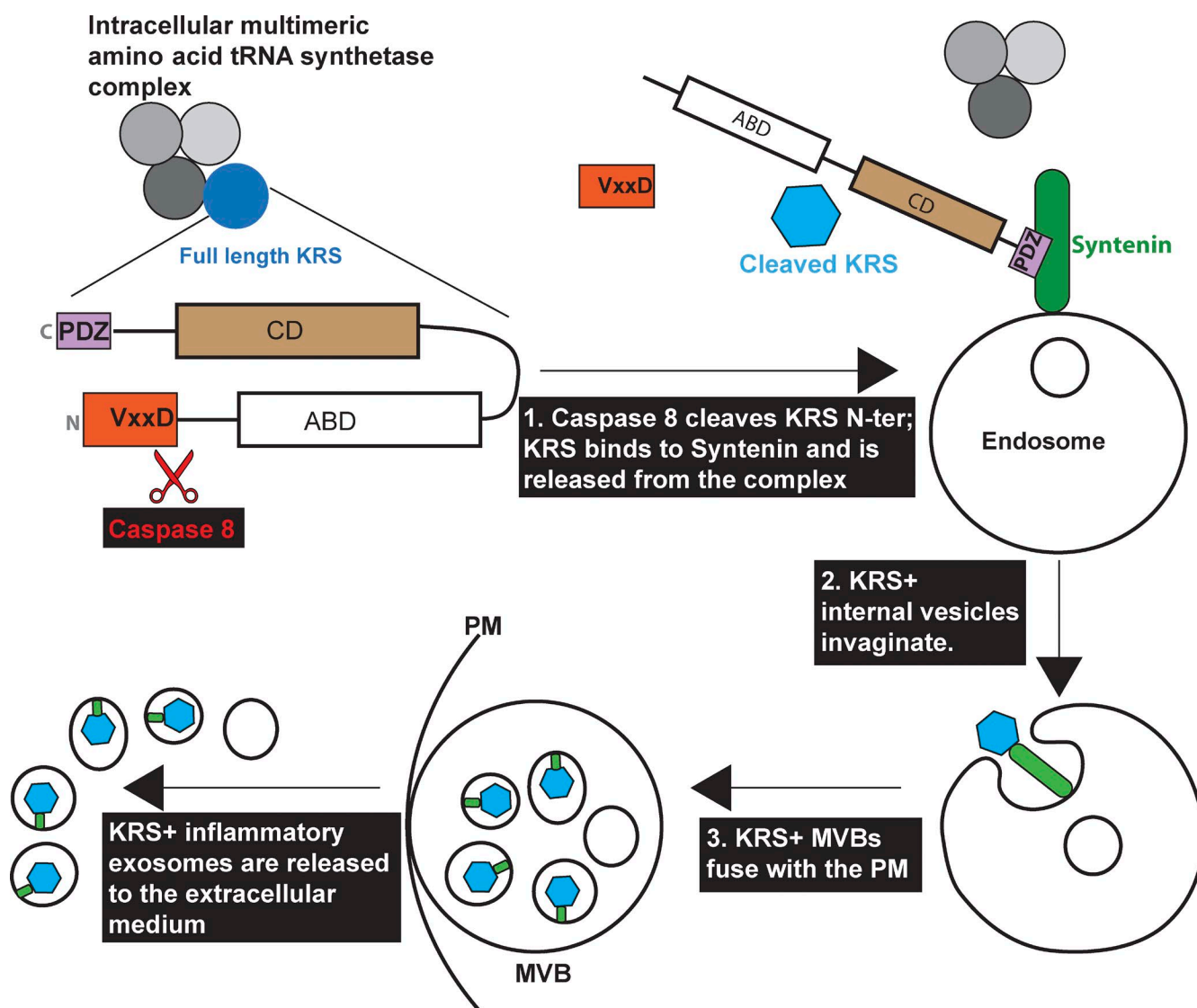


Figure 1. **Model of KRS exosome release to the extracellular medium.** KRS (dark blue circle) is normally found in an intracellular multimeric tRNA synthetase complex, where it performs its housekeeping function of binding lysine to tRNA. The C terminus PDZ (violet box) is probably buried and does not bind syntenin (green bar). (1) Upon activation (caused by inflammation or/and starvation), caspase-8 cleaves the first 12 amino acids at the N terminus (orange box) of KRS. The PDZ domain of cleaved KRS (light blue octagon) can bind to syntenin that is localized to the endosomal membrane. (2) Syntenin mediates the incorporation of cleaved KRS in internal vesicles. (3) Multi-vesicular bodies (MVBs) fuse to the plasma membrane (PM). (4) KRS-positive inflammatory exosomes are released to the extracellular medium. ABD, anticodon binding; CD, catalytic domain.

et al., 2010; Ostrowski et al., 2010). Third, depletion of several ESCRT components also led to a strong reduction in KRS release. Fourth, immuno-EM revealed that KRS is localized in the lumen of small cup-shaped vesicles of 100 nm. Collectively, these four lines of evidence are consistent with the release of KRS in exosomes (Fig. 1). Interestingly, the presence of KRS in the extracellular medium appears to be entirely accounted for by its incorporation in exosomes, suggesting that this is the sole mechanism for KRS release.

KRS release in exosomes depends on its binding to syntenin that guides it to ALIX. Accordingly, syntenin depletion inhibits KRS release. Kim et al. (2017) show that KRS binds syntenin via its extreme C-terminal PDZ domain (i.e., the last five residues of KRS), and KRS lacking this PDZ domain is not released. Furthermore, replacing the amino acid sequence of canonical KRS PDZ domain with alanines also prevents KRS release. This suggests that syntenin acts as a re-

ceptor for the incorporation of KRS in exosomes through the binding of its PDZ domain.

What regulates the specific binding of KRS to syntenin? One clue to this question came from the observation that the form of KRS present in the medium has a slightly faster mobility than intracellular KRS, which is suggestive of a cleavage event. Both the cleavage and release of KRS in exosomes require the N terminus of KRS to be free (not tagged). Using a series of N-terminal truncations, the cleavage site was mapped between residues 12 and 13 (Fig. 1). Interestingly, the amino acid sequence around position 12 (VxxD) is consistent with a caspase cleavage site, and the substitution of aspartate 12 with alanine completely abrogated KRS cleavage and its release in exosomes. Pharmacological inhibition of caspase activity confirmed the role of caspases in KRS release, and RNAi screening of specific caspases revealed a key role for caspase-8 in this process. Depletion of caspase-8 led to a drastic reduction

of KRS release, and conversely, caspase-8 overexpression led to an increase. Interestingly, the release of KRS in exosomes was strongly stimulated by starvation, and this treatment also increased caspase-8 activity, whereas the activity of other caspases was not affected. Strikingly, Kim et al. (2017) found that the cleavage of the N terminus of KRS by caspase-8 modulates the binding of the C terminus PDZ domain of KRS to syntenin. This suggests that KRS cleavage leads to a major change in its conformation, which may expose the PDZ domain. Accordingly, syntenin binding to KRS is concomitant with the dissociation of KRS from the multi-tRNA synthetase complex to which it usually belongs inside cells. Therefore, it appears that caspase-8-mediated cleavage leads to a change in the conformation of KRS, allowing it to bind syntenin and be released from the complex. This ensures the specific incorporation of cleaved KRS into internal vesicles and its release in exosomes (Fig. 1).

KRS is known to bind to macrophages and monocytes and induce them to start producing TNF, a key step in inflammation and macrophage migration (Park et al., 2005). Kim et al. (2017) then addressed whether the KRS-containing exosomes are inflammatory when presented to macrophages both in vitro and in vivo when injected in mice. Indeed, they found that not only TNF but also CRG-2, IL6, and MMP9 were released by macrophages, and these become migratory when treated with KRS-containing exosomes. Importantly, this response is very similar to that elicited by naked KRS. This suggests that KRS release in exosomes triggers inflammation and may help create a microenvironment for cancer cell survival and potentiate metastasis.

However, many questions about the process of KRS release remain to be answered. First, as mentioned above, starvation stimulates exosome production and the incorporation of KRS therein. Although the mechanism of this stimulation is not well understood, it is interesting that exosome release is stimulated by stress, as this is also observed for many other UPS pathways (Rabouille, 2017). Second, syntenin is proposed to be the KRS receptor mediating its incorporation into internal vesicles of endosomes. Syntenin itself is recruited to the endosomal membrane through its binding to the proteoglycan syndecan (Baietti et al., 2012). It will therefore be interesting to show whether KRS release in exosomes is also dependent on syndecan. Third, KRS in exosomes is as potent as naked KRS in stimulating the inflammatory response and migration of macrophages. It seems likely that KRS is released from exosomes, but the mechanism of this release needs to be better understood. Are exosomes taken up in macrophage endosomes, and is the membrane dissolved there? Does KRS become membrane free in the medium?

KRS is now established as a caspase-8 substrate. Caspase-8 normally exists as a pro-caspase-8 enzyme that is cleaved and

activated by inflammation. Of note, this caspase-8 activity does not appear to be triggering apoptosis. This suggests that a small level of inflammation around the cancer cells activates caspase-8, which triggers more inflammation through the release of KRS. This in turn activates pro-IL1 $\beta$  in macrophages and leads to the release of mature IL1 $\beta$ , leading to full-blown inflammation and potentially creating a niche for cancer cell survival and proliferation. Interestingly, mature IL1 $\beta$  is also unconventionally secreted (by type I and III depending on the stress; Rabouille, 2017), suggesting that the inflammation triggered by cancer cells might be the sum of several unconventional secretion modes.

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