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Vault RNA emerges as a regulator of selective autophagy

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ABSTRACT

The selective autophagic receptor SQSTM1/p62 ushers cargo to phagophores, the precursors of autophagosomes, and serves as a platform for autophagy initiation. We discovered that SQSTM1 is an RNA-binding protein that interacts with vault RNAs. Vault RNAs are small non-coding RNAs found in many eukaryotes and transcribed by POLR3 (RNA polymerase III). The levels of *VTRNA1–1* (vault RNA 1-1) regulate SQSTM1-mediated autophagy and ubiquitin aggregate clearance. Vault RNA interferes with oligomerization of SQSTM1, which is in turn critical for its autophagic function. Our study uncovered a novel mode of regulation of a protein's activity by RNA, termed riboregulation.

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SQSTM1/p62 (sequestosome 1) is a prototypical member of selective autophagy receptors, which can bridge phagophore membranes to polyubiquitin-marked cargos. Oligomerization of SQSTM1 is a key feature enabling the formation of large SQSTM1 structures that can tether to Atg8-family proteins and serve as a platform for the assembly of autophagy initiation complexes.

RNA-binding proteins regulate the fate of RNAs at all stages of their lifetime. Recently, a number of complementary biochemical methods were developed to define the RNA-binding proteomes of cell culture models, tissue samples and whole organisms. A unifying feature of these datasets is the presence of many proteins that were hitherto unknown to play roles in RNA biology. Intriguingly, a mass spectrometry-based analysis indicated that SQSTM1 binds RNA via its zinc finger domain [1]. Many routes of intracellular RNA degradation were previously described, including degradation of RNA by lysosomes. The presence of SQSTM1 among the RNA-binding proteins sparked our attention, as no autophagic receptor was previously shown to directly interact with RNA, and we therefore speculated that SQSTM1 might mediate lysosome-dependent RNA degradation.

To identify SQSTM1-bound RNAs we first covalently stabilized SQSTM1-RNA complexes *in cellulo* by 254 nm UV-C crosslinking, and performed SQSTM1 immunoprecipitation followed by next-generation sequencing. Surprisingly, we found that SQSTM1 binds a narrow set of RNAs including a few mRNAs and some tRNAs. The most enriched RNA class that interacts with SQSTM1 is the vault RNAs (*VTRNAs*). Vault RNAs form a family of small non-coding RNAs transcribed by the POLR3. Vault RNAs were identified as components of ribonucleoprotein complexes termed 'vaults', found in a variety of eukaryotes, including humans. The function of vaults and vault RNAs remains unclear despite their conservation and abundance in all human tissues. We employed a variety of *in vitro* and *in cellulo* methods to show that SQSTM1 preferentially binds *VTRNA1-1*. However, contrary to our initial expectations, the absence of SQSTM1 does not have an impact on the levels of *VTRNA1-1*, suggesting that the interaction between SQSTM1 and the RNA is unlikely to mediate autophagic degradation of vault RNAs.

Because the autophagic function of SQSTM1 is well defined, we investigated whether the interaction of vault RNA and SQSTM1 affects autophagy. We found that experimental perturbation of the levels of *VTRNA1-1* affected SQSTM1-dependent autophagy under steady-state conditions. Removal of *VTRNA1-1* increases LC3B conjugation, decreases SQSTM1 levels, and augments the LC3B-SQSTM1 interaction. Moreover, the lack of *VTRNA1-1* is beneficial for the SQSTM1-dependent clearance of ubiquitin aggregates upon inhibition of the proteasome. These effects are independent of the activity of MTOR which functions as an upstream effector of autophagy. Thus, we concluded that *VTRNA1-1* functions as an inhibitor of SQSTM1's activity.

Provided that the levels of vault RNA have an impact on SQSTM1's function, what physiological conditions could affect the levels of *VTRNA1-1*? We noticed a drop in total *VTRNA1-1* levels upon culture of cells in amino acid-free medium. The drop in total levels is not observed for other members of the vault RNA family or other tested POLR3 transcripts. Concomitantly, amino acid starvation induces a gradual decrease of RNA-bound SQSTM1. Removal of *VTRNA1-1* prior to starvation exacerbates the colocalization of SQSTM1 and LC3B puncta, suggesting that the lack of *VTRNA1-1* enables an increase in SQSTM1 activity.

Our initial mass spectrometry analysis implicated SQSTM1 amino acid residues involved in the interaction with RNA. We engineered an RNA binding-deficient version of SQSTM1 and confirmed that this mutagenesis indeed reduces RNA binding. We noticed that UV-C treatment stabilizes SQSTM1 oligomers,

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observed as 'ladders' in SQSTM1 western blots. Interestingly, the RNA binding-deficient variant of SQSTM1 shows pronounced oligomer formation compared to the WT SQSTM1 counterpart. These data suggested regulation of SQSTM1 oligomerization as a possible molecular mechanism of vault RNA function.

The N-terminal PB1 domain of SQSTM1 mediates its homo-oligomerization, which is required for its autophagic activity and is sufficient to promote SQSTM1 localization to phagophores. As expected, we observed increased SQSTM1 oligomerization following amino acid starvation *in cellulo*. The ZZ domain, which is juxtaposed C-terminally to the PB1 domain, emerged recently as a hotspot for the regulation of the PB1 domain activity. A ZZ domain chemical ligand, which was previously shown to induce SQSTM1 oligomerization and an increase in autophagic flux, indeed intensifies LC3B conjugation in *VTRNA1-1* knockout cells compared to the WT controls. Taken together, our experimental data suggest that *VTRNA1-1* inhibits SQSTM1 activity through the regulation of SQSTM1's oligomerization state.

Our study unveiled a defined function of a vault RNA, and established an unprecedented example of regulation of a protein's activity by RNA, a process that we refer to as 'riboregulation'. Structural details of *VTRNA1-1* binding to SQSTM1 and the consequence of this binding on SQSTM1 oligomerization are expected to shed light on the molecular details of this regulation in the future.

What could be the overarching biological principle of autophagy regulation by vault RNA? Because the role of vault RNA association with vault particles as well as the function of vault particles *per se* is understudied, the connection between vaults and autophagy deserves further exploration. Intriguingly, infection by influenza A and Epstein-Barr viruses trigger high expression of vault RNAs. Also, mice lacking vaults are more susceptible to infection with *Pseudomonas aureginosa*, an obligatory extracellular pathogen that is targeted by phagocytic cells of the host immune system. Autophagy plays an important role in resolving these infections. We wonder whether the vault RNA–SQSTM1 regulatory axis could represent an important facet of coupling intracellular immunity and autophagy.

Disclosure statement

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Reference

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