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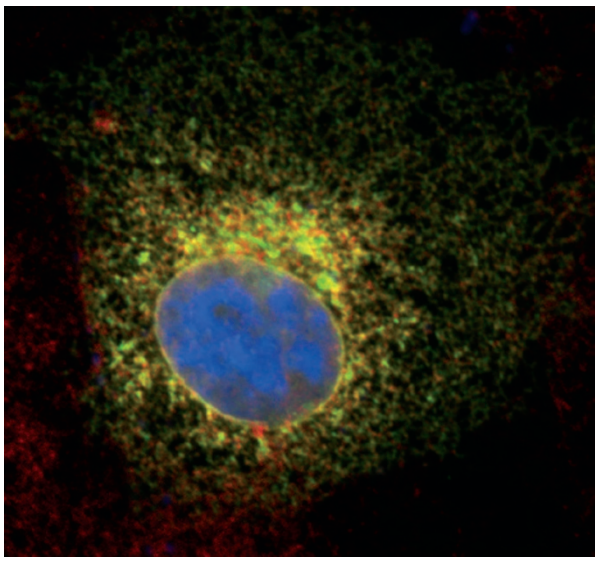
PROTEIN METABOLISM

The benefits of inactivity

> iRhoms can effectively sequester a substrate away from its intramembrane protease.

Intramembrane proteases catalyse irreversible cleavage of proteins, so it is crucial to limit their action. It is unclear how this is achieved, but there is some evidence that physically separating the substrates from these proteases is important. Zettl *et al.* have found that, in the case of Rhomboid proteases, they may be kept in check by closely related but enzymatically inactive Rhomboids (iRhoms), which can divert substrates into the endoplasmic reticulum-associated degradation (ERAD) pathway.

The iRhoms have previously been characterized by phylogenetic analysis as a subfamily of Rhomboid-like proteins that are conserved across metazoans. They lack two key catalytic residues but instead have an invariant Pro residue nearby. Consistent with this, Zettl *et al.* showed that iRhoms from *Drosophila melanogaster*, mice and humans do not have proteolytic activity *in vitro* and that introducing the iRhom-specific Pro into active Rhomboids was sufficient to abolish proteolysis.



Mouse inactive Rhomboid protease (green) localizes to the endoplasmic reticulum (red); DNA is shown in blue. Image courtesy of K. Strisovsky and M. Zettl, Medical Research Council Laboratory of Molecular Biology, Cambridge, UK.

To elucidate the biological role of iRhoms, the authors focused on the *D. melanogaster* iRhom, which is expressed in the central nervous system during embryogenesis and in adults. Flies that lack iRhom develop normally overall but show unusual patterns of sleep — a phenotype previously observed when the epidermal growth factor receptor (EGFR) pathway is overactive. Given that Rhomboids are known to target EGFR ligands in *D. melanogaster*, the authors speculated that iRhom may also affect this process. Indeed, they showed that in the developing wing and eye, iRhom shows synergistic genetic interactions that indicate that iRhom inhibits EGFR signalling.

So how does iRhom act at the cellular level? In COS7 cells, tagged *D. melanogaster* and mammalian iRhoms localized to the ER and could prevent the cleavage of EGFR ligands by Rhomboid proteases. Importantly, the presence of iRhoms correlated with reduced levels of *D. melanogaster* or mammalian ligands, which was proteasome dependent. Proteins in the ER can be targeted for proteasomal degradation by ERAD. The authors saw that tagged EGF and iRhom proteins co-immunoprecipitate, indicating that iRhom might target EGF towards the ERAD pathway. Analysis of EGF turnover in a pulse–chase experiment also supported this idea and, *in vivo*, components of the ERAD pathway showed genetic interactions with EGFR signalling in the developing eye. In cell lines lacking endogenous Rhomboid activity, the expression of an iRhom still downregulated EGFR ligands, again suggesting that iRhoms act directly on these substrates rather than indirectly through a Rhomboid protease.

Thus, iRhoms provide a unique means of regulating the very proteins that they evolved from, the active Rhomboid proteases: by binding to Rhomboid substrates at the ER and targeting them for degradation, iRhoms can effectively sequester a substrate away from its intramembrane protease. This also illuminates a new way to limit EGFR signalling in *D. melanogaster*, by crosstalk with ER quality control. It will be exciting to see how broad the repertoire of iRhoms is and how their target specificity is determined. Such pseudoenzymes are prevalent, and this may be a first glimpse of the many physiological roles that they have.

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ORIGINAL RESEARCH PAPER Zettl, M. *et al.* Rhomboid family pseudoproteases use the ER quality control machinery to regulate intercellular signalling. *Cell* **145**, 79–91 (2011)