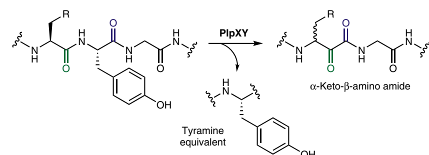
PEPTIDE MODIFICATION

Specialized splicing

Science **359**, 779–782 (2018)



Credit: AAAS

Natural ribosomes incorporate only α-amino acids into peptides and proteins; though β-amino acids have been found in some natural products, they are the result of nonribosomal peptide and polyketide biosynthesis. Morinaka et al. now report the discovery of a new group of ribosomally synthesized and post-translationally modified natural products that contain β-amino acids as the result of a post-translational splicing reaction. A radical-SAM enzyme termed a splicase (PlpX), in conjunction with a putative leader peptide recognition protein (PlpY), excises a tyrosine-derived tyramine equivalent from the precursor peptide and then rejoins the backbone to form an α-keto-β-amino amide. Although the reaction requires the presence of a Tyr–Gly motif at the splice site, PlpX is otherwise somewhat tolerant to mutations to the native precursor peptide sequence, including at the residue immediately N-terminal to the splice site. Modification at this position thus enables the generation of peptides containing various β-amino acids. The ketoamine moiety is also useful for bioorthogonal labeling, as it can be converted to an oxime by methoxyamine or directly conjugated to a thiosemicarbazide-labeled fluorophore. The PlpXY system could be used to generate libraries of compounds for drug screening, which the authors demonstrated by biosynthesizing an analog of the hepatitis C protease inhibitor boceprevir. *CD*

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LIPIDS AND MEMBRANES

A 'cis'ted localization

eLife **7**, e35588 (2018)

Phosphatidylinositol-4-phosphate (PtdIns4*P*) is involved in regulating vesicular traffic as well as nonvesicular lipid transport at the plasma membrane (PM), late endosomes/lysosomes, and the Golgi through binding to regulatory proteins. The phosphatase SAC1 regulates PtdIns4*P* levels and is localized primarily to the endoplasmic reticulum (ER). SAC1 has been proposed to regulate PtdIns4*P* levels at the PM, the Golgi and the endosomes through a 'trans' mechanism whereby SAC1 localizes to membrane-contact sites (MCSs) between the ER and these compartments. Evidence is also available for a 'cis' mechanism of SAC1 action on the PM- and Golgi-localized PtdIns4*P* that invokes separate lipid-transfer proteins. The transfer proteins move lipids from the ER to the PM or the Golgi and back-traffics a PtdIns4*P* molecule down its steep chemical gradient to the ER, where it can be degraded by SAC1. To distinguish between 'cis' and 'trans' SAC1 mechanisms, Zewe et al. first inhibited SAC1 and found that PtdIns4*P* accumulated at the ER. Furthermore, a fluorescently tagged SAC1 does not enrich at the ER-PM MCS. These results, a series of experiments using a chemically induced dimerization approach to induce ectopic ER–PM MCS, and measurements of PtdIns4*P* abundance together suggest a 'cis' mechanism for SAC1 and exclude a 'trans' mechanism, defining a critical role for SAC1 in driving countertransport of lipids against their chemical gradients. *MB*

https://doi.org/10.1038/s41589-018-0029-0

PROTEIN ENGINEERING

Mix and match

Nat. Struct. Mol. Biol. **25**, 289–296 (2018)

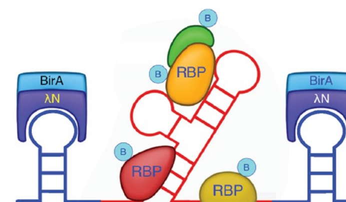
Nanobodies have found widespread use in structural biology, in cell biology and as therapeutics, but almost all are still derived from alpaca and llama immunizations, which is time consuming and expensive. To overcome these limitations, McMahon et al. developed a surface-display platform for the isolation of selective nanobodies from yeast cells. The authors first generated a synthetic nanobody library based on sequence alignments of structurally characterized nanobodies. The designed nanobodies were then fused to a long linker region and a C-terminal anchor sequence that tethers them to the yeast cell wall for surface display while the target protein was labeled with a fluorescent dye. Yeast cells expressing the nanobodies were then incubated with the labeled protein, which was followed by repeated selection rounds with anti-fluorophore magnetic microbeads or fluorescence-activated cell sorting. Single yeast colonies were sequenced after the final selection round. The identified nanobodies were then expressed and purified from *Escherichia coli*. The authors demonstrate that conformationally selective G-protein-coupled receptor-binding nanobodies can be isolated, which is useful for functional studies and crystallization. The method is versatile, because either purified or nonpurified antigens can be used, and other selection procedures may also be implemented. This rapid nanobody discovery platform could make nanobody preparation more easily accessible and cost-effective. *KK*

https://doi.org/10.1038/s41589-018-0028-1

RNA–PROTEIN INTERACTIONS

RaPID hookup

Nat. Methods **15**, 207–212 (2018)



Credit: *Nat. Methods*

Current approaches to studying RNA–protein interactions, such as the RNA electrophoretic mobility shift assay (EMSA) or UV or formaldehyde crosslinking combined with immunoprecipitation, are mostly performed in vitro, so they may not reflect physiologically relevant interactions in cells. Ramanathan et al. developed an approach called RaPID that enables detection of RNA–protein complexes without the need for crosslinking. RaPID utilizes a protein composed of the N terminus of *Escherichia coli* BirA*, a promiscuous biotin ligase, fused to the λN peptide, which specifically binds to BoxB stem loops. Incubation of the peptide with an RNA motif flanked by BoxB stem loops in cells in biotin-containing media enables biotinylation of proteins that are bound to the RNA motifs. These labeled proteins can then be detected with streptavidin capture and mass spectroscopy analysis. RaPID confirmed known RNA–protein interactions such as IREB2 binding to the iron-responsive element RNA motif and identified new interactions such as RC3H1 binding to the SM1v RNA motif, which is enriched in breast cancer patients. Sequence homology analysis guided the modification of *Bacillus subtilis* biotin ligase to BASU, which exhibited rapid biotin kinetics and improved signal-to-noise ratio compared to BirA*. Overall, the RaPID approach provides a useful alternative to EMSA and will enable detection of genetic or small-molecule-mediated disruption of RNA–protein interactions. *GM*

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