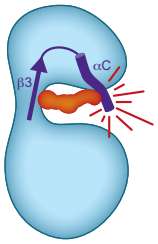
KINASE REGULATION

Shortening the loop

Cancer Cell http://dx.doi.org/10.1016/j.ccell.2016.02.010 (2016)



ELSEVIER

The C helix of many protein kinases, including BRAF, has distinct conformational states that are linked to enzyme activity. Inactive kinases have an outward αC conformation; kinase activation shifts αC to an inward orientation, allowing a catalytic bridge to form with the β3 strand. This knowledge has informed the development of kinase inhibitors that specifically target the outward (vemurafenib) or the inward conformation (AZ-628) of the C helix. Recent large-scale sequencing of samples from patients with pancreatic tumors revealed a recurring five-amino-acid (NVTAP) deletion in the BRAF β3-αC loop, but the effects on kinase activity and inhibitor responsiveness were not known. Foster *et al.* found that cells expressing this shortened loop (BRAFΔNVTAP) exhibited constitutive signaling activity and were sensitive to AZ-628 but resistant to vemurafenib, suggesting that the deletion variant is in the inward conformation. This was verified by structural analysis of BRAFΔNVTAP complexed with AZ-628, which showed that the αC conformation was predominantly in the inward orientation and that steric restraints in the shortened loop prevented a shift to the outward conformation, explaining the inability of αC-out inhibitors to interact with the BRAFΔNVTAP. Interestingly, analogous β3-αC mutations in EGFR and HER2 were also resistant to C-helix-out inhibitors such as lapatinib. These results suggest that future inhibitor discovery should include careful consideration of mutational and conformational status. *GM*

RNA TRAFFICKING

RCas9 lights the way

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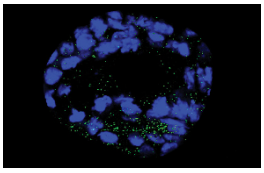
While fluorescent protein fusions are widely used to visualize the cellular localization of proteins, very few methods exist to follow RNA processing and trafficking. Nelles *et al.* now engineer the CRISPR-Cas9 system, a pervasive tool for targeted genome editing, into a system capable of tracking specific RNAs within living cells. In this RCas9 system, the authors fuse an inactivated Cas9 nuclease (dCas9) to a fluorescent protein, equip it with a single-guide RNA (sgRNA) that targets dCas9 to a specific mRNA sequence, and include a "PAMmer" synthetic oligonucleotide that binds the target mRNA and also prevents DNA targeting. The RCas9 approach was applied to track the movement of several mRNAs, with results that correlated closely with those of RNA fluorescence *in situ* hybridization (FISH) assays. RCas9–mRNA complexes were sufficiently stable to allow visualization of mRNA transport from the nucleus to the cytoplasm or to follow the trafficking of endogenous mRNAs, such as *ACTB*, to stress granules after induction of cellular stress. Further, RCas9 expression and RNA binding do not perturb the cellular abundance or function of target mRNAs. Taken together, these results validate RCas9 as a useful tool for RNA visualization that may be readily adapted to probe RNA processing and function within cells. *TLS*

INFLAMMATION

Dietary stress relief

Nature **531**, 523–527 (2016)



NATURE

The kinase GCN2 is a sensor of amino acid depletion in cells and triggers the integrated stress response (ISR). Ravindran *et al.* set out to define its role in modulating immune responses during amino acid depletion. Using mice deficient for GCN2, the authors found that GCN2 expression both in epithelial cells and in antigen-presenting cells of the immune system was necessary to protect mice from symptoms of colitis, including gut inflammation and increased intestinal permeability in response to an inducer of inflammatory bowel disease. The authors found that the inflammation-suppressing effects of GCN2 were dependent on the downstream kinase eiF2α and occurred through a mechanism dependent on the induction of enhanced autophagy. Consistent with a role for autophagy in the control of reactive oxygen species (ROS), a well-known trigger of inflammasomes, the authors observed increased ROS in the colon and small intestine of the *Gcn2* knockout mice during inflammation. Neutralization of ROS resulted in diminished inflammation. They further demonstrated enhanced inflammasome activation in *Gcn2* knockout mice, and they found that genetic ablation of the inflammasome molecule Asc in the *Gcn2* knockout mice negated the enhanced inflammation. Finally, amino acid deprivation could protect mice from the symptoms of colitis via a GCN2-dependent mechanism, implicating GCN2 in a function that couples sensing of amino acids with control of intestinal inflammation. *MB*

PROTEIN SYNTHESIS

Taming transmembrane proteins

J. Am. Chem. Soc. **138**, 3553–3561 (2016)

Membrane-spanning proteins are notoriously challenging to study owing to their hydrophobic nature and tendency to form aggregates; this makes them difficult to purify and recalcitrant to solid-phase peptide synthesis (SPPS), the primary tool for the production of proteins with precise post-translational modifications or site-specific labeling. To facilitate the synthesis of membrane-spanning proteins by SPSS, Zheng *et al.* developed a removable backbone modification (RBM), consisting of one or more arginine residues, that is straightforward to install during the synthesis of transmembrane regions and facile to remove once synthesis of the peptide is complete. The RBM tag is compatible with a variety of amino acid sequences and improves handling of the synthesized peptide by increasing its aqueous solubility and disrupting aggregation. Using this method, the authors achieved the synthesis and purification of a selectively ^{15}N-labeled p7 ion channel from hepatitis C virus, enabling them to study its ligand interactions by NMR, as well as the synthesis of the four-transmembrane *Escherichia coli* protein EmrE. While this method is currently limited to use in small- to medium-length peptides, the compatibility of this RBM with native chemical ligation suggests that it could in future be used to synthesize larger membrane-spanning proteins. *CD*

Written by Mirella Bucci, Caitlin Deane, Grant Miura & Terry L. Sheppard