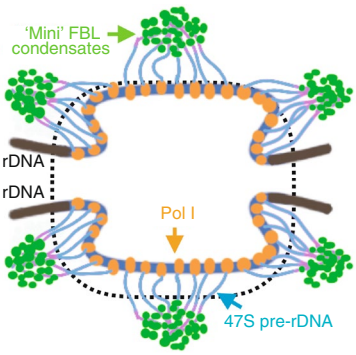
RNA-BINDING PROTEINS

Disorder guides order

Mol. Cell https://doi.org/10.1016/j.molcel.2019.08.014 (2019)



Credit: Elsevier

The most abundant RNA components in cells are ribosomal (r)RNA, which are transcribed and processed within the nucleolus. However, the rapid processing of pre-rRNAs makes it challenging to study the underlying mechanisms. Using multiple high-resolution optical imaging approaches, Yao et al. found that pre-rRNA processing factors, including FBL, formed clusters in dense fibrillar components (DFC) surrounding the active rDNA and Pol I complex localized at the border of fibrillar centers of nucleoli. FBL underwent phase separation via self-association of its GAR domain and guided nascent 47S pre-rRNA into DFC through interaction with the 5′ terminus of pre-rRNA. The length of intrinsically disordered regions (IDRs) in GAR domains is correlated to its self-association ability and is associated with pre-rRNA processing. Interruption of the interaction between FBL and pre-rRNA impairs the formation of FBL clusters. Replacement of the GAR domain with different IDRs rescued FBL-knockout-induced abnormality in pre-rRNA sorting and processing. This study reveals the roles of IDRs in sub-organelle organization and deepens our understanding of connections between spatial organization and biological function. *YS*

https://doi.org/10.1038/s41589-019-0398-z

ION CHANNELS

An inside job

Cell **178**, 1362–1374 (2019)

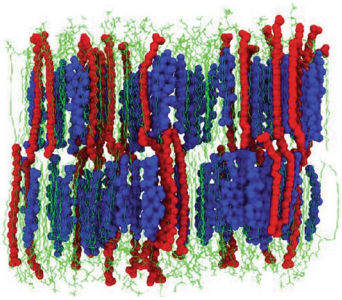
Most known agonists of the TRPA1 ion channel are reactive electrophiles that covalently modify cysteine residues in a cytoplasmic domain of the channel called the allosteric nexus. Although some non-covalent TRPA1 agonists have been identified, these are typically of low potency and specificity, limiting their use for analgesic drug development. To identify new TRPA1 agonists, Lin King et al. screened a collection of arachnid venoms and identified a scorpion disulfide-linked peptide toxin, WaTx (wasabi receptor toxin), as exhibiting potent and selective activity with TRPA1 in cells but lacking electrophilic character. Patch-clamp and FRET assays indicated that WaTx crosses membranes to access the cytoplasmic side of the TRPA1 channel, likely enabled by a basic patch at one end of the peptide's helical structure. Surprisingly, a series of mutagenesis and protein-interaction experiments localized the WaTx-binding site to the same allosteric nexus targeted by electrophilic agonists. However, channel activation kinetics and permeability differ upon binding of WaTx versus electrophilic agonists, suggesting that they operate through distinct mechanisms of action. The discovery of a potent non-electrophilic TRPA1 agonist could provide a route to further understanding and treatment of pain. *CD*

https://doi.org/10.1038/s41589-019-0395-2

MEMBRANES

Poked to death

ACS Chem. Biol. https://doi.org/10.1021/acschembio.9b00616 (2019)



Credit: ACS

Fatty acids consisting of linear alkyl chains with various degrees of unsaturation can act as bioactive lipids through covalent modification of signaling proteins and modulation of the biophysical properties of membranes. The abundance of very-long-chain fatty acids (VLCFAs) of 20 or more carbons increases during necroptosis, a form of programmed cell death. Parisi et al. found that blocking the accumulation of VLCFAs, which is known to lessen necroptotic cell death, protected cells from membrane permeabilization, whereas increasing VLCFAs caused permeabilization. Consistently, VLCFA C24:0, but not long-chain FA C16:0, induced permeabilization of liposomes that mimic the lipid composition of the plasma membrane. Given the relatively low amounts of VLCFAs that accumulate during necroptosis, the authors speculated that cells must have a mechanism to concentrate the VLCFAs at the membrane to reach sufficient levels to cause permeabilization and promote necroptosis. Indeed, they found that a C20:0-based FA could covalently modify specific proteins, promoting their membrane localization. Inhibiting these modifications led to increased cell viability during necroptosis. These results suggest that VLCFAs promote necroptosis by disrupting membranes through a direct mechanism, as well as an indirect one via protein acylation. *MB*

https://doi.org/10.1038/s41589-019-0397-0

CANCER THERAPY

Mistaken identity

Sci. Trans. Med. **11**, eaaw8412 (2019)

There is growing evidence that the failure of clinical trials for new drugs may be due to off-target effects. To determine whether this is a general phenomenon, Lin et al. performed a systematic CRISPR–Cas9-based competition screen generating null mutations in the known targets for ten cancer drugs. They found that loss of these cellular targets did not impair cancer cell growth, which differed from previous findings using RNA interference. The authors suggested that the previous RNA interference findings were a result of off-target toxicity. In addition, inhibitors against these five cellular targets were responsive in cells deficient for the respective targets, confirming that the cytotoxicity of these inhibitors was due to off-target inhibition. Lin et al. further investigated OTS964, a reported inhibitor of PBK, and identified OTS964-resistant clones. Sequencing analysis revealed missense mutations in the cyclin-dependent kinase member CDK11B that conferred resistance to OTS964. Loss of CDK11B function was found to prevent mitotic progression, suggesting that CDK11B may be the critical target of OTS964. Overall, the findings by Lin et al. stress the importance of target validation of cancer drugs before progressing to clinical trials. *GM*

https://doi.org/10.1038/s41589-019-0396-1

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