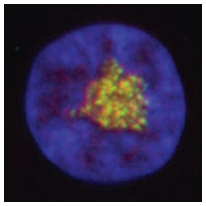
CANCER THERAPEUTICS

Blowing up the nucleolus

Cancer Cell **25**, 77–90 (2014)



ELSEVIER

In cancer cells, nucleolar activity and ribosomal RNA (rRNA) synthesis by RNA polymerase I (Pol I) are increased. Therefore, compounds that can reduce rRNA synthesis could be considered for therapeutics. The small molecule BMH-21 reduces viability in a wide variety of cancer cell lines and blocks *in vivo* tumor growth in a p53-independent manner, but the mechanism of action has remained unclear. Peltonen *et al.* observed that BMH-21 is a DNA-intercalating agent that binds to GC-rich regions of DNA. In particular, BMH-21 exhibited specific binding to rDNA, resulting in the rapid inhibition of Pol I transcriptional activity. This loss of transcription was associated with increased detection of nucleolar stress in cells characterized by structural changes in the nucleolus and mislocalization of nucleolar proteins such as nucleolin and nucleophosmin. In addition, BMH-21 treatment resulted in the polyubiquitination and proteasomal degradation of RPA194, the largest catalytic subunit of Pol I. The loss of RPA194 expression prevented the Pol I complex from binding to rDNA and correlated well with decreased cancer cell survival. Taken together, this work suggests that inhibiting components of the ribosomal RNA synthesis machinery may be an effective strategy for future cancer therapeutics. *GM*

GPCRS

Guilt by dissociation

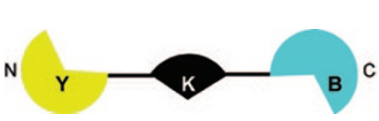
J. Biol. Chem. **289**, 1271–1281 (2014)

Signals downstream of G protein–coupled receptors (GPCRs) are transduced and amplified by G protein heterotrimers, consisting of subunits α, β and γ. In the canonical G protein cycle, activation of GPCRs causes exchange of Gα-bound GDP for GTP and Gα dissociation from Gβγ dimers. This part of the cycle is less clear for the $G_{i/o}$ family of G proteins because of conflicting *in vitro* and *in vivo* results, but three models have been debated that differ mainly by whether the $G\alpha_{i/o}$ subunit dissociates from Gβγ and to what extent. Therefore, it has been unclear whether a rearranged heterotrimer ($G\alpha_{GTP}G\beta\gamma$) or a dissociated trimer ($G\alpha_{GTP}$, Gβγ) is the active GTP-bound form capable of transmitting the GPCR signal to effectors. To reconcile these discrepancies, Bondar and Lazar used two-photon polarization microscopy, which can report on changes in protein-protein interactions and protein conformational changes of fluorescently labeled proteins (FPs) or native proteins. They also looked at localization of GRK3, a kinase associated with GPCRs whose binding to Gβγ is favored upon dissociation of activated $G_{i/o}$, and looked at regulation of GIRK channels downstream of it. They found that at endogenous concentrations, 85–95% of nonmodified $G_{i/o}$ molecules dissociate upon activation, suggesting that dissociated Gα and Gβγ are the major activated form that mediates downstream signaling in a natural setting. However, some FP-tagged $G_{i/o}$ constructs were able to activate downstream signaling without dissociation, suggesting that $G_{i/o}$ dissociation is not strictly required for activity. *MB*

PROTEIN FOLDING

The inside scoop

J. Am. Chem. Soc. **136**, 858–861 (2014)



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Protein folding studies have typically focused on those sequences that are under thermodynamic control, meaning they fold into a single structure regardless of the starting conditions. However, proteins may also fold under kinetic control, where one of multiple possible final structures is selected on the basis of early conformational biases. Previous studies have shown that co-translational folding can stabilize particular folding intermediates, suggesting this process could similarly influence the populations of final structures. To test this, Sander *et al.* designed a 'YKB' polypeptide from known split fluorescent protein fragments: the central K fragment can complement either the Y or B fragment to form a fluorescent protein, but with different fluorescent properties for the resulting YK and KB structures, which provides a facile readout of relative populations *in vivo*. Refolding YKB *in vitro* resulted in a 1:1 ratio of YK and KB products, whereas cellular translation biased the pool toward YK structures, as would be expected if Y and K were able to fold while B was still being synthesized. The authors similarly observed that altering the translation rate—controlled via insertion of rare and thus more slowly processed codons—further increased the YK:KB ratio, but only if the exchanged codons were in the nucleotide sequence encoding the B domain. These results raise new questions regarding folding *in vivo* and provide a new strategy for future research. *CG*

RECEPTORS

Plants pick up ATP

Science **343**, 290–294 (2014)

ATP is essential for cellular energy metabolism, but accumulating evidence suggests that it also acts as an extracellular signaling molecule in mammals through two classes of membrane-bound receptors. Plants are known to release ATP in response to wounding, and elevated extracellular ATP triggers responses such as calcium ion release in plant cells. However, homology-based searches have failed to find ATP receptor candidates in plants. Choi *et al.* now report the identification of the first ATP receptor in *Arabidopsis thaliana*. A genetic screen of mutagenized *Arabidopsis* seedlings identified two mutants, referred to as "Does not respond to nucleotides 1" (*dorn1-1* and *dorn1-2*), which lack a cytoplasmic calcium release response in the presence of extracellular ATP. The *dorn1* point mutations both mapped to a gene encoding lectin receptor kinase-I.9 (LecRK-I.9), a transmembrane protein with a putative extracellular ligand-binding domain and an intracellular kinase domain. The mutations blocked LecRK-I.9 kinase activity *in vitro* and *in planta*, but ATP signaling could be rescued by ectopic expression of wild-type LecRK-I.9. Competition assays using radiolabeled nucleotides and 8-azido-ATP revealed that ATP is the preferred nucleotide ligand of DORN1. Transcriptomic analysis revealed that the ATP-dependent changes in gene expression, which were blocked by *dorn1* mutations, overlapped significantly with gene expression changes that occur in response to plant wounding, suggesting that DORN1 may mediate the physiological response to the wounding-induced release of ATP in plants. *TLS*

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