LEUKEMIA

PHFriends with RAR

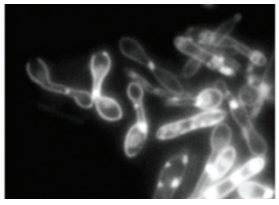
Cancer Cell **23**, 376–389 (2013)

All-*trans* retinoic acid (ATRA) is a targeted therapy for acute promyelocytic leukemias (APL) caused by gene fusions involving retinoic acid receptor α (RARα). The underlying mechanistic basis for ATRA action and resistance to ATRA in these cancers is not well understood. Because RARα fusions are known to aberrantly recruit epigenetic enzymes to DNA, Arteaga *et al.* performed a systematic biochemical screen for interactions between RARα fusions and the JmjC family of lysine methyltransferases, identifying PHF8 as a specific interacting factor. Using chromatin immunoprecipitation assays, the authors showed that PHF8 is localized to the promoters of RARα fusion target genes and that this association correlates with the modification of histone methylation marks and increased expression of these genes. To demonstrate a relationship between ATRA and PHR8, the authors showed that overexpression of catalytically active PHF8 in cells resistant to ATRA restored sensitivity to the drug selectively in cells expressing RARα fusions *in vitro* and *in vivo*. RNAi-mediated suppression of PHF8 in ATRA-sensitive cells rendered the cells resistant to the drug. In addition, the authors showed that ATRA treatment alters the promoter occupancy of PHF8 depending upon serine phosphorylation. Taken together, these data provide a molecular mechanism to explain the mode of action of ATRA in APL and point to possible mechanisms for ATRA resistance. *AD*

PEPTIDOGLYCAN

Rip it up

PLoS Pathogens **9**, e1003197 (2013)



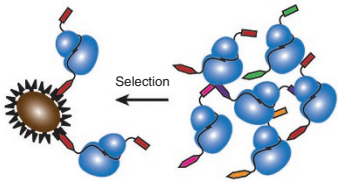
MICHAEL CHAO

Peptidoglycan hydrolases are required to degrade the bacterial cell wall during cell division but must be controlled to avoid excess hydrolysis and loss of cell integrity. Recent work has pointed to functional interactions between peptidoglycan hydrolases and other proteins, and the hydrolase RipA is known to contain a protease-sensitive loop, but the overall regulatory mechanisms remain unclear. Chao *et al.* now show that RipA from *Mycobacterium smegmatis* is controlled by two post-translational processing mechanisms. The authors first confirmed that overexpression of RipA is toxic to cells. Inhibition of peptidoglycan formation by addition of the β-lactam meropenem was similarly toxic but not in RipA-depleted cells, strengthening previous evidence of a link between peptidoglycan synthesis inhibition and dysregulation of hydrolase activity. RipA transcription was fairly constant, ruling out this possible regulatory mechanism. Instead, the authors observed cleavage of the protein—most likely within the flexible loop—to form smaller constructs associated with the cell wall. The authors confirmed that proteolysis is necessary for function by showing that an inactivated C408A mutant could compete for the unknown processing enzymes. Similarly, introduction of the homologous *M. tuberculosis* protein, which is not processed by the *M. smegmatis* machinery, was nontoxic, whereas a truncated construct was toxic. The data support a zymogen model in which RipA is secreted from cells, binds interaction partners and is cleaved to initiate hydrolase activity. *CG*

PROTEIN INTERACTIONS

PLATO's molecular dialog

Nat. Biotechnol., published online 17 March 2013; doi:10.1038/nbt.2539



NATURE BIOTECHNOLOGY

The identification of molecules that interact with and affect the function of proteins is important for understanding biomolecular networks. To identify protein-binding molecules in a high-throughput fashion, Zhu *et al.* now report parallel analysis of translated open reading frames, or PLATO. To prepare a library of cells for PLATO, the authors expressed mRNAs that are tethered to the proteins they encode using a known technique called ribosomal display. Following *in vitro* transcription and translation, the proteins were screened for binding to an immobilized bait molecule; candidate binding proteins were enriched by quantitative real-time PCR or deep DNA sequencing. The authors applied PLATO to discover new protein-protein interactions, protein targets for antibodies and protein targets for small molecules. These analyses identified new interacting proteins for LYN kinase, revealed likely epitopes in TRIM family proteins on the basis of their interaction with autoantibodies in the cerebral spinal fluid of patients with autoimmune disease and confirmed known kinase targets for the small-molecule receptor tyrosine kinase inhibitor gefitinib. Taken together, these data demonstrate that PLATO is a versatile high-throughput platform for the identification of protein-binding molecules. *AD*

DRUG DISCOVERY

Stolen isoprenoids

Antimicrob. Agents Chemother. **57**, 1804–1814 (2013)

Cryptosporidium parvum is a gastrointestinal parasite that causes deadly recurrent diarrhea with limited treatment options. To find new candidate drugs that are cost effective, efficacious, safe and US Food and Drug Administration (FDA) approved, Bessoff *et al.* developed a semiautomated microscopy-based assay to monitor *C. parvum* growth in cultured human ileocecal cells. Screening a collection of 727 FDA-approved drugs and drug-like molecules for inhibitors of *C. parvum* growth in this assay identified 19 anticryptospoidial compounds in three chemical families, represented by pyrimidine analogs, tetracycline analogs and statin drugs that inhibit HMG-CoA reductase, the rate-limiting step in the mevalonate (MVA) pathway. The MVA pathway is one of two routes to isopentyl pyrophosphate (IPP) and dimethylallyl diphosphate, essential precursors of many biological molecules, including prenylated proteins. Many pathogens do not have the MVA machinery and instead use an alternative (MEP) pathway to generate the precursors. Although *C. parvum* has neither pathway, the authors found in a bioinformatics analysis that the parasite does encode enzymes associated with the use of the isoprenoid precursors, suggesting it uses isoprenoids synthesized by the host. Consistent with this idea, they found that addition of exogenous IPP relieved the inhibition of *C. parvum* growth by the statin itavastatin. These results suggest that inhibition of the host's HMG-CoA reductase by statins is detrimental to *C. parvum* because of its reliance on exogenously derived isoprenoid precursors. *MB*

Written by Mirella Bucci, Amy Donner, Catherine Goodman & Terry L. Sheppard