TOOLS

A nice complement

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Functional complementation—in which polypeptide fragments are combined to regenerate active proteins, such as with split GFP—can provide a background-free readout for various cellular functions. However, identifying protein fragments that are sufficiently stable to withstand separation and that generate a functional change upon recombination is not straightforward. Pandey *et al*. now simplify the search for suitable constructs by using transposon mutagenesis to facilitate construction of protein libraries. The authors used either the IAAL-E3 and IAAL-K3 peptides or CheA and CheY proteins as fusion partners for fragments of near-infrared fluorescent protein (IFP) to both stabilize the isolated constructs and drive assembly. Testing 1,760 mutants of each system led to the identification of 13 sites where IFP could be split and successfully recombined. The relative fluorescence of the assembled fragments ranged from ~15–150% of the signal generated by the wild-type IFP control, with the fusion partners required for optimal assembly. The mutations were found on the linker between IFP's two domains and secondary structure elements on either side of the linker but were not near the chromophore binding site, spurring questions regarding the mechanism of complementation. These results provide new tools for interrogation of cellular systems and useful guidelines for further fragment discovery. *CG*

EPIGENETICS

Bumping into BET inhibitors

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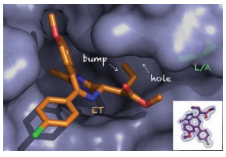


JQ1 and I-BET are chemical probes that inhibit bromo and extra-terminal (BET) proteins, which are transcriptional regulatory proteins that contain bromodomains, 'readers' of acetylated lysines within chromatin. These compounds potently bind bromodomains of BET-family proteins but are pan-selective within the BET family. Baud *et al.* now report a chemical genetics approach that identifies an orthogonal and more selective BET-bromodomain inhibitor. Sequence alignments and structure-guided analyses identified a conserved leucine residue in BET-bromodomains involved in inhibitor-protein interactions; mutation of this leucine to the smaller alanine (L/A) created a 'hole' that did not interfere with BET protein stability or function but could accommodate an inhibitor with a complementary hydrophobic 'bump'. An ethylated I-BET analog (ET) was designed and synthesized, and crystallographic analysis of an ET–L/A mutant bromodomain complex identified a specific contact between the ethyl group of ET and the mutant alanine residue. Differential scanning fluorimetry and isothermal titration calorimetry measurements revealed that ET is up to 540-fold selective for L/A mutants relative to wild-type bromodomains, including in BET proteins with tandem bromodomains. This selectivity persists in cells and was applied to demonstrate that targeting the first bromodomain of a BET protein is sufficient to displace the protein from chromatin. The new study shows that the bump-and-hole approach offers potential as a tool to enhance our understanding of chromatin biology. *TLS*

MICROBIOMES

Gut persuasions

Cell **159**, 1–14 (2014)

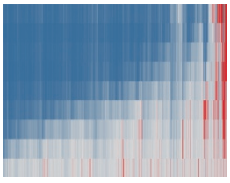
Identifying the microbes that inhabit a particular environment as well as competition among different cohabitating species can help in understanding microbial invasion mechanisms and in treating diseases associated with dysbiosis of the microbiota in the human gut. To study how microbes colonize a gut environment and to characterize the conditions under which different microbial species can live (their 'fundamental niche'), Seedorf *et al.* performed several transplantation experiments where they introduced microbiota isolated from different non-mice habitats (xenomicrobiota) into germ-free mice. These xenomicrobiota included those isolated from human and zebrafish gut, human tongue and skin, termite hindgut and a non-animal (soil) community. Subsequent characterization by 16S rRNA sequencing of the colonized microbiomes revealed that the mouse gut is within the fundamental niches of a greater proportion of bacterial taxa from non-mice gut environments compared to those from non-gut habitats. Nevertheless, further experiments examining biomass levels, carbohydrate and bile acid metabolism and co-housing of animals to report on microbiota function showed that most bacterial phylotypes cannot realize this niche in a gut harboring indigenous microbiota. In addition, experiments where the germ-free recipient mice lacked mature T and B immune cells allowed the authors to conclude that there is greater selective pressure on xenomicrobiota upon transplantation into the foreign host compared to the indigenous mouse gut microbiota. These results highlight the dynamic interplay among diverse species that can be exploited for understanding colonization and for generating next-generation probiotics. *MB*

TARGET VALIDATION

Bringing the heat

Science **346**, 1255784 (2014)

SCIENCE



By measuring the specific temperature required to dissociate an interaction between a ligand and its target, the thermal shift assay has been used to detect and quantify drug-target engagement in cells. However, the application of this approach to provide unbiased identification of cellular targets of a drug remained a challenge. To address this, Savitski *et al.* combined the thermal shift assay with high-resolution quantitative MS to determine the effects of a drug on the thermal profile of the cellular proteome. The authors added either vehicle or drug to cells and heated lysates or intact cells across a range of ten different temperature points. Proteins at a particular melting temperature begin to denature and aggregate. These samples were labeled with a different TMT10 isotope tag for LC/MS/MS analysis to measure the fraction of soluble proteins at each temperature, producing a characteristic melting curve for each protein. Ligand binding to a protein creates a thermal shift in the curve, increasing the overall T_m for the protein. The authors tested whether their system could detect new off-targets of clinical kinase inhibitors such as vemurafenib and alectinib. Interestingly, both compounds targeted ferrochelatase (FECH), an enzyme in the heme biosynthesis pathway. FECH deficiency is associated with protoporphyria, a painful photosensitivity disorder. As vemurafenib and alectinib treatment are also known to cause phototoxicity, these findings suggest that the off-target inhibition of FECH may explain these side effects. Taken together, this technique offers an approach to provide proteome-wide identification of potential off-targets of drugs. *GM*

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