CELL BIOLOGY

Conditional nanobody tools

Nanobodies that are stable only in the presence of their antigen can be harnessed to manipulate or detect antigen-expressing cells.

Manipulating specific cell types in the mouse can be difficult, as mouse lines that allow the necessary manipulations may not be available. But if lines expressing the popular marker GFP in the cell type of interest exist, GFP-specific nanobodies may provide an inroad into this problem. Nanobodies are small, single-chain antibodies that can be expressed in cells by means of, for example, viral delivery. To manipulate cellular processes, scientists have used combinations of nanobodies to assemble active protein complexes or to reconstitute split effector domains in the presence of a specific antigen such as GFP. "We can use existing GFP expression patterns in transgenics to create specificity for the biological perturbations," explains Harvard Medical School researcher Constance Cepko.

Although existing nanobody approaches work well, they require several components, which challenged Cepko and her team, including co–first authors Jonathan Tang and Eugene Drokhlyansky, to come up with a simpler method for manipulating cells depending on GFP expression. They wanted to create a single reagent that would be active only in GFP-expressing cells. "We explored the idea that we could take a nanobody, fuse it to some kind of functional domain like Cre and have it be degraded unless GFP was present," explains Cepko.

To generate such a conditionally stable nanobody, the researchers created a viral library of mutant GFP nanobodies fused to blue fluorescent protein and developed a screening procedure that allowed them to identify infected cells that exhibited blue fluorescence only when GFP was present. "And that worked incredibly well," says Cepko. She thinks that the principle of the screen can be applied to other types of protein binders as well. Importantly, however, it might not be necessary to repeat the screen for most other nanobodies, as the mutations that conferred antigen-dependent stability to the GFP nanobody are in highly conserved regions of the protein, and the researchers showed that these mutations can be grafted onto other nanobodies.

Cepko and her team used the destabilized nanobodies to induce cell-type-specific perturbations in GFP-expressing mouse lines. They fused the destabilized GFP nanobodies to

SENSORS AND PROBES

MAKING SENSE OF NAD$^+$ SUBCELLULAR LOCALIZATION

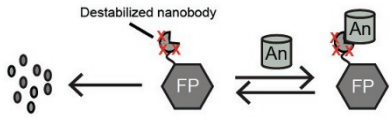
A fluorescent NAD$^+$ biosensor targeted to specific cellular compartments detects local fluctuation in NAD$^+$ concentration.

Nicotinamide adenine dinucleotide (NAD$^+$) is best known as a cofactor in metabolic processes, but lately it has come into the limelight as a substrate for sirtuins and poly(adenosine diphosphate-ribose) polymerases (PARPs), enzymes that regulate protein deacetylation and DNA repair. The subcellular colocalization of NAD$^+$-generating and NAD$^+$-consuming enzymes suggests that the activity of NAD$^+$-dependent enzymes may be regulated by localized fluctuation in NAD$^+$, but this was not experimentally proven. To measure subcellular pools of free NAD$^+$, a team from the Vollum Institute in Oregon led by Richard Goodman has now generated a cellular-compartment-specific NAD$^+$ biosensor.

Cellular levels of NAD$^+$ are maintained by the cooperative action of nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide mononucleotide adenyltransferases (NMNATs), which recycle components of NAD$^+$ breakdown to regenerate the active form of the coenzyme. The final step of NAD$^+$ biogenesis is catalyzed by three enzyme isoforms, each specific to a cell compartment—nucleus, cytoplasm or mitochondria.

To study NAD$^+$ levels, Goodman's team fused Venus fluorescent protein to two NAD$^+$ binding sites from bacterial DNA ligase (Cambronne *et al.*, 2016). The resulting

Cre or Flp recombinases, effectively turning GFP expression patterns into recombinase expression patterns and allowing them to manipulate gene expression or neural activity in specific cells.



A nanobody–effector domain fusion (nanobody-FP) is stable in the presence of cognate antigen (An) and is degraded in its absence. Reproduced with permission from Figure 1 in Tang *et al.* (2016) (https://creativecommons.org/licenses/by/4.0/legalcode).

The destabilized nanobodies can also detect the presence of proteins independently of reporter gene expression. For example, Cepko and her team collaborated with Behzad Eteman and Jonathan Li to detect HIV-1. "Detection of viral epitopes intracellularly in live cells is something that is very hard to do," says Cepko. As a demonstration, the researchers generated destabilized nanobodies against an HIV-1 capsid protein and fused them to blue fluorescent protein. The researchers were then able to detect and isolate live HIV-1 infected cells by their blue fluorescence. Their approach was highly sensitive owing to the lack of background fluorescence in uninfected cells. The lack of background from the use of destabilized nanobodies is an advantage of this approach, as stable nanobodies fused to a fluorescent protein will give signal even in cells that do not express the targeted epitope.

Cepko envisions that there will be a wide variety of applications for destabilized nanobodies. Depending on the availability of specific nanobodies and their mutation into conditionally stable versions, these tools could detect specific protein phosphorylation in live cells. Alternatively, as fusions to Cas9, these destabilized nanobodies could allow CRISPR/Cas9-mediated gene editing only in cells that express the target gene, as the tools would be degraded in cells that were already edited and no longer expressed the target gene, thereby potentially reducing the risk of off-target effects.

Cepko thinks that the destabilized nanobodies could be used to manipulate specific cells even in genetically untractable organisms. "If you can deliver genes to an organism, you can then use this approach to get specificity in cell-type manipulations," she says.

Nina Vogt

RESEARCH PAPERS

Tang, J.C.Y. *et al.* Detection and manipulation of live antigen-expressing cells using conditionally stable nanobodies. *eLife* **5**, e15312 (2016).

chimeric protein can bind NAD^+ but cannot consume it. When it is attached to NAD^+, its fluorescence is quenched at only one of two excitation peaks, allowing measurements of free NAD^+ to be normalized to sensor expression level. Precise quantification of NAD^+ levels using sensor targeted to different compartments of mammalian cells showed that mitochondria contain more than twice as much free NAD^+ as other compartments. The authors also show that gene silencing and pharmacologic inhibition of NAMPT led to a decrease in NAD^+ concentration in all compartments studied, but depletion of mitochondrial NAD^+ occurred at a slower rate. By systematically silencing the expression of each NMNAT isoform, the team demonstrated that the cytoplasmic and nuclear pools are readily exchangeable, whereas at least two mechanisms maintain mitochondrial NAD^+: import from the cytoplasm and NAD^+ biogenesis by mitochondrial isoform NMNAT3.

Some NAD^+ can be phosphorylated into $NADP^+$, which has different roles in metabolism. A team from the University of Toronto recently generated a $NADP^+$ biosensor that can offer new insights into a variety of diseases and conditions, highlighting the importance of small-molecule probes in metabolic studies (Cameron *et al.*, 2016). Similarly, as NAD^+-consuming enzymes are implicated in immunity, metabolism and lifespan control, the NAD^+ sensor holds promise to advance the study of disease and aging.

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RESEARCH PAPERS

Cambronne, X.A. *et al.* Biosensor reveals multiple sources for mitochondrial NAD^+. *Science* **352**, 1474–1477 (2016).

Cameron, W.D. *et al.* Apollo-$NADP^+$: a spectrally tunable family of genetically encoded sensors for $NADP^+$. *Nat. Methods* **13**, 352–358 (2016).