CELL BIOLOGY

Not the same

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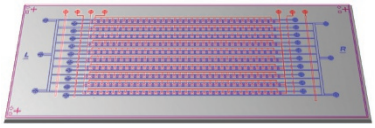
JACS



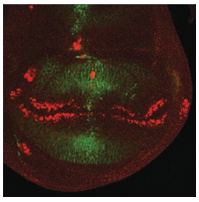
Analyses of biological processes at the single-cell level have revealed that cells with identical genotypes can behave quite differently. This means that the average values obtained from measurements of ensembles of cultured cells may not accurately represent the phenotypes of many of the individual cells. This heterogeneity can make it especially difficult to study complex systems like tumors, which usually contain many metabolically dissimilar cell types. Xue *et al.* have constructed a microfluidic device to help them study cellular heterogeneity, and this 'lab-on-a-chip' can simultaneously analyze metabolites and proteins from a single cell. Each one of these devices (see image) contains 310 1.5-nanoliter-sized chambers, enabling the user to perform ~100 single-cell assays and the appropriate controls at the same time. A single cell is loaded into each nanochamber; the cell is then lysed, and the elements of the cell lysate are then captured by a miniaturized antigen array patterned into each chamber, where the amounts of seven selected proteins, cAMP, cGMP, glutathione and glucose are determined. The authors used this microfluidic device to study single GBM39 neurosphere tumor cells before and after treatment with erlotinib, an epidermal growth factor receptor (EGFR) inhibitor. The data obtained from the single-cell experiments confirmed what had been observed in bulk solution: that erlotinib inhibits EGFR phosphorylation, glucose uptake and the activity of hexokinase 2. Further analysis of the data indicated that there were two distinct phenotypes of tumor cells: 80% of the cells had high glucose uptake, low cAMP levels and low cGMP levels, whereas the remaining cells had the opposite metabolic phenotype. The authors suggest that it will be relatively straightforward to increase the number of proteins and metabolites that the microfluidic device can measure, and such a device could be used to better understand how cell-to-cell metabolic differences can result in major changes in cellular behavior. *JMF*

WNT SIGNALING

Trimming the fat

Nature **519**, 187–192 (2015)
Dev. Cell **32**, 719–730 (2015)

NATURE



Wnts are secreted signaling molecules that are covalently linked to the monounsaturated fatty acid palmitoleic acid, which directly inserts into the hydrophobic cleft of the Frizzled receptor. Wnts also interact with particular heparin sulfate proteoglycans called glypicans to mediate extracellular distribution and signaling efficacy of the Wnt ligands. There are a number of secreted inhibitors, such as Notum, that modulate Wnt signaling. Notum was initially thought to act as a phospholipase that hydrolyzes the GPI anchor of glypicans to restrict the extracellular distribution of Wnts through glypican-mediated sequestration. Kakugawa *et al.* and Zhang *et al.* tested this putative mechanism and found that Notum does not cleave the GPI anchor of glypicans. Instead, as shown by Kakugawa *et al.*, Notum interacts with the sulfated glycosaminoglycan chains. Loss of glypican activity reduces Notum-mediated inhibition and cell-surface localization. To identify the target of Notum's activity, Kakugawa *et al.* determined the crystal structure of human Notum, revealing a canonical α/β hydrolase fold and two distinct ligand-binding pockets. One pocket contained a heparin saccharide–binding site, which was confirmed through SPR assays to mediate the direct glypican-Notum interactions, whereas the other was a large hydrophobic pocket that could potentially accommodate unsaturated fatty acids such as palmitoleic acid. Another crystal structure from Kakugawa *et al.*, showing Notum bound to a palmitoleoylated Wnt7a peptide, confirmed that the fatty acid occupied the hydrophobic pocket, with the ester bond located close to the catalytic center. Both groups of authors suspected that Notum is a carboxylesterase that cleaves the ester bond connecting palmitoleate to Wnt. Kakugawa *et al.* used mass spectrometry analysis whereas Zhang *et al.* used palmitic analogs to detect Notum-mediated cleavage of Wnt's palmitoleic acid linkage. Finally, Zhang *et al.* found that Wnt3a lacking palmitoleic acid formed soluble oligomers linked by intermolecular disulfide bonds that were unable to interact with the Frizzled receptor. Overall, these findings identify Notum as a novel extracellular deacylase. *GM*

RIBOSWITCHES

Manganese management

Mol. Cell **57**, 1110–1123 (2015)

Riboswitches are RNA motifs that adaptively bind metabolites and couple ligand recognition to regulation of gene expression. Though the three-dimensional structures and the gene-regulatory mechanisms of numerous riboswitch families have been characterized, the physiological ligands of certain riboswitches remain unknown. For instance, the identity of the ligand for the abundant *yybP-ykoY* riboswitch, which was first described in *Bacillus subtilis* over a decade ago, has not been established. Now Price *et al.* provide evidence that the *yybP-ykoY* riboswitch is a Mn^{2+}-responsive transcriptional ON switch. Based on recent data showing that *yybP-ykoY* is found upstream of genes linked to Mn^{2+} trafficking, the authors hypothesized that the riboswitch may be a Mn^{2+} sensor. *In vitro* transcription assays with *yybP-ykoY* from *Lactococcus lactis* revealed that Mn^{2+}, but not other transition-metal ions, relieved basal transcriptional termination, an effect that was confirmed in cells using a reporter gene construct. X-ray crystallographic and mutational analysis alongside chemical probing data of the *L. lactis* riboswitch revealed a riboswitch architecture based on two coaxially stacked helices organized by a four-way junction that, in the presence of Mn^{2+}, creates two phosphate-rich metal-binding sites; one site could be assigned as a Mg^{2+} binding pocket, while the other serves as the Mn^{2+}-sensing site and features an octahedral coordination geometry at the metal ion that includes a 'soft' N7 purine ligand donated by A41. Finally, exploring the link between the riboswitch and the physiological effects of elevated Mn^{2+} concentrations, the authors showed that expression of *yybP-ykoY*–linked YoaB from *L. lactis*, a putative P-type ATPase, was able to rescue sensitivity to elevated Mn^{2+} concentrations in *B. subtilis*, providing support for the function of YoaB as a Mn^{2+} efflux pump. Taken together, the combined biochemical and structural data establish Mn^{2+} as the physiological ligand of the *yybP-ykoY* riboswitch. *TLS*

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