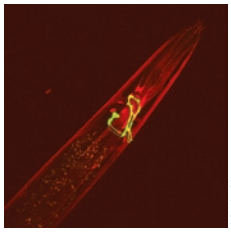
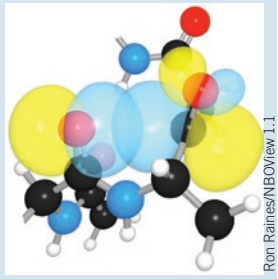
Nod to a new neurotransmitter


Mark Alkema

Classical biogenic amines such as dopamine and serotonin are well-established regulators of animal behavior. The animal nervous system also produces a variety of other biogenic amines, but the biological roles of these trace amines remain unclear. Pirri *et al.* now show that tyramine functions as a neurotransmitter that mediates motor functions in the nematode *Caenorhabditis elegans*. Wild-type worms propel themselves by sinusoidal body movements, which are accompanied by side-to-side head motion. However, when organisms are prodded or treated with exogenous tyramine, they rapidly reverse while suppressing head motion. To understand these escape responses, Pirri *et al.* carried out a genetic screen and found that animals with mutations in *lgc-55* are resistant to the tyramine-induced movement phenotype. Further experiments established that LGC-55 is a tyramine-responsive chloride-selective ion channel expressed in neurons that regulate locomotion and head movement. The current results provide a compelling case that tyramine is a neurotransmitter that activates LGC-55 channels and is directly involved in motor responses in *C. elegans*. Given the similarities between the escape responses of *Drosophila* and *C. elegans*, the study also raises the intriguing possibility that tyramine's role as a neurotransmitter may extend to other invertebrates or higher organisms. (*Neuron* **62**, 526–538, 2009) *TLS*

Protein principles


Ron Raines/NBOView 1.1

Carbonyl-carbonyl interactions along a protein chain represent an energetically small but frequent contribution to the overall noncovalent forces in protein structures. Though some work has suggested that the basis for this attraction is through orbital overlap, yielding an $n \rightarrow \pi^*$ interaction, other results have pointed toward a dipole-dipole interaction. It is also possible that the carbonyl-carbonyl contact is mediated by Coulombic attraction between the partially positive carbon atom and the partially negative oxygen atom. To resolve this matter, Choudhary *et al.* used chemical principles to generate paired dipeptide analogs that could discriminate between the potential interaction modes. The authors used proline as a building block to position two carbonyls close together in space. By changing one of the carbonyls to a thiocarbonyl and monitoring conformational exchange by NMR, the authors were able to rule out a charge-charge interaction as being responsible for the carbonyl contact. Additionally, by introducing a fluorine atom as an electron-withdrawing group to bias the proline ring pucker, the authors concluded that this noncovalent force cannot be attributed to a dipole-dipole interaction, and is indeed best described as an $n \rightarrow \pi^*$ interaction. X-ray diffraction and computational analysis of the compounds further supported this conclusion. As carbonyl-carbonyl interactions are found in α-helices and other common secondary structures, this result advances our understanding of the fundamental forces that govern protein folding. (*J. Am. Chem. Soc.* **131**, 7244–7246, 2009) *CG*

Easy as 1, 2, 3

Counting algorithms are important components of digital devices and are essential to many cellular functions. Engineering synthetic counting systems into cells may equip scientists with tools to regulate more complex cellular behaviors. Friedland *et al.* now report two classes of synthetic gene networks that count induction events by small molecules. The first type of network uses an initial construct that constitutively expresses mRNA for T7 RNA polymerase (T7 RNAP), the translation of which is repressed by a *cis* riboregulator sequence. A first pulse of arabinose leads to expression of a transactivating RNA that relieves translational repression and produces T7 RNAP. T7 RNAP then transcribes a second GFP construct, and a second arabinose pulse leads to translation of GFP. Fluorescent quantification revealed that two arabinose pulses, or 'counts', were required to produce substantial amounts of GFP, and this approach was also extended to count to three by inserting a second RNA polymerase gene construct in series before GFP. The second network type also enabled counting to two or three, but is based on constructs in which recombination modules were manipulated by expression of recombinase enzymes under the control of small-molecule inducers. Though still rudimentary by computational standards, these new counting schemes in *Escherichia coli* offer promise for manipulating cellular function on controlled time scales. (*Science* **324**, 1199–1202, 2009) *TLS*

Written by Mirella Bucci, Catherine Goodman, Joanne Kotz & Terry L. Sheppard

DAG nabs microtubules

When stimulated by antigens, T cells secrete soluble factors that can be cytotoxic or cell-stimulatory. To help ensure that bystander cells are not affected, factor secretion is directed toward the 'immunological synapse', the junction between the T cell and the antigen-presenting cell (APC). Directional secretion is mediated by the polarization of the microtubule organizing center (MTOC) in response to T-cell stimulation. Early T-cell signaling events also promote the recruitment of many effector enzymes, including phospholipase C-γ (PLC-γ), which generates diacylglycerol (DAG). Microtubules radiate from the MTOC with the 'minus' ends in, thus implicating dynein (a 'minus end'–directed microtubule motor) in promoting the orientation at the immunological synapse. To explore the coupling between early T-cell signals to dynein recruitment and reorientation of the MTOC, Quann *et al.* used a previously described photoactivation assay for MTOC orientation towards the APC to test the roles of PLC-γ and its products. Experiments with small-molecule effectors suggested that DAG is involved in guiding microtubule movement and maintaining a polarized MTOC. Using TIRF microscopy, the authors then showed that DAG recruitment to the immunological synapse preceded dynein recruitment, which preceded MTOC reorientation. Further experiments using inhibitors of DAG metabolic enzymes and a caged version of DAG confirmed a role for localized DAG in driving MTOC polarization and the subsequent cytotoxic effects of the T cells. Though the exact mechanism of DAG function is unknown, its accumulation at immunological synapses may recruit dynein to promote MTOC reorientation. (*Nat. Immunol.*, published online 10 May 2009, doi:10.1038/ni.1734) *MB*

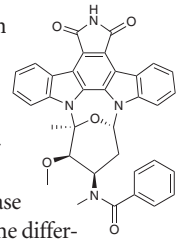
Simplifying synthesis

Facile access to complex natural products and diverse compound libraries remains a central challenge in organic synthesis. One strategy to enable synthetic transformations is 'one-pot' protocols, in which several reagents or catalysts are combined in a single flask to perform multiple reactions. Jiang *et al.* use this approach to develop a new enantio- and diastereoselective method to access 4,5-disubstituted isoxazoline-N-oxides. Other strategies to obtain these versatile building blocks required significant amounts of reagents or were not selective. In this case, key α-bromo or epoxide intermediates allowed the creation of N-oxides with two or three chiral centers; the further elaboration of these compounds into sphingosine and amino sugar derivatives demonstrates their synthetic utility. In an alternative approach to generate complex chiral molecules, Steinhardt and Vanderwal report the serendipitous discovery that certain aldehydes undergo a reaction cascade to form polycyclic lactams. While investigating the mechanistic pathway to generate α,β-unsaturated amides from Zincke aldehydes, the authors observed an unexpected reaction product. Exploration of different starting materials allowed the creation of several bi- and tricyclic scaffolds, each of which contains multiple groups suitable for further reactions; in particular, the use of indole-containing starting materials provided a building block related to indole alkaloids. These combined studies highlight new chemistry that will be useful for a broad range of research. (*Angew. Chem. Int. Ed.*, published online 13 May 2009, doi:10.1002/anie.200901446; *J. Am. Chem. Soc.*, published online 18 May 2009, doi:10.1021/ja902439f) *CG*

NMR screening goes cellular

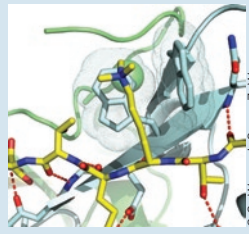
In vitro NMR-based screening has proven to be a powerful approach for identifying fragments or small molecules that bind to biomolecular targets. In contrast, cell-based screening has the advantage of looking at inhibition in a more physiologically relevant context; however, deconvoluting the target of a cellular screen can be challenging. Xie *et al.* now take advantage of recent advances in in-cell NMR structure determination to conduct a cellular screen with molecular resolution. The authors overexpressed uniformly ^{15}N-labeled FKBP along with its unlabeled binding partner, the rapamycin binding domain of mTOR (FRB), in *Escherichia coli* and detected formation of the weakly interacting complex. Addition of the small molecule rapamycin to these cells resulted in formation of the more tightly binding ternary complex and chemical shift perturbations consistent with the previously characterized rapamycin binding site. Subsequent titration of ascomycin, a natural product that binds FKBP competitively with rapamycin, resulted in displacement of rapamycin from the complex and binding of ascomycin to an overlapping but more extended site on FKBP. Using this system, the authors screened a 289-member dipeptide library and identified peptides that disrupted the FKBP-FRB complex in cells and showed activity in a functional yeast assay. These proof-of-principle results suggest that in-cell NMR will be useful for identifying small-molecule leads and for structurally characterizing small molecule–protein complexes within living cells. (*J. Med. Chem.*, published online 7 May 2009, doi:10.1021/jm9000743) *JK*

Endowing endoderm



Directing the differentiation of embryonic stem cells (ESCs) into particular cell types is a major goal for understanding the development of various tissues and for generating cells for disease modeling and treatment. The differentiation of ESCs *in vitro* is poorly controlled, requiring multiple steps and often resulting in nonhomogenous populations. To simplify this process, many researchers have sought small molecules that can control lineage commitment in ESCs. Zhu *et al.* have now screened 20,000 compounds for those that could induce differentiation of mouse and human ESCs into definitive endoderm progenitors—cells that ultimately go on to form internal organs including the liver, pancreas and lung. One compound, stauprimide, increased the percentage of cells that express endoderm markers at the expense of markers of other lineages. The cells could be further differentiated into pancreatic precursors or hepatocytes by incubation with combinations of defined growth factors. Stauprimidine eliminated the need for fetal bovine serum in the standard differentiation protocol towards definitive endoderm; however, the protocol still required activin A, which is known to be involved in endoderm specification. Besides definitive endoderm, stauprimide could also promote ESC differentiation into neural progenitor cells and mesoderm when combined with known lineage specification cues, suggesting that stauprimidine primes ESCs for differentiation. The authors identified stauprimide's target as NME2, a c-Myc transcription factor known to be involved in maintaining the undifferentiated ESC state. Stauprimide downregulates c-Myc transcription, providing a mechanism for stauprimide's priming action. (*Cell Stem Cell* **4**, 416–426, 2009) *MB*

Oncogene PHounD



G.G. Wang, J. Song, Z. Wang, D.J. Patel & C.D. Allis

Plant homeodomain (PHD) fingers recognize different methylation states of histone H3 Lys4 (H3K4). Mutations in the PHD finger of ING1 have been implicated in multiple cancers. In addition, chromosomal translocations that fuse the PHD finger–containing protein JARID1A, which recognizes the trimethylated site H3K4me3, to NUP98, a common leukemia fusion partner, have been found in people with acute myeloid leukemia (AML). However, direct evidence that the histone reading activity of PHD fingers contributes to oncogenesis has been lacking. Wang *et al.* have now found that a PHD finger–containing (but not a PHD finger–lacking) isoform of the JARID1A-NUP98 fusion protein functions as a leukemic oncogene in cells and in mice. Structural analysis showed that the PHD finger binds the trimethylated lysine residue in a hydrophobic pocket bordered by two tryptophan residues. Mutation of these tryptophan residues disrupted H3K4me3 binding *in vitro* and in cells and prevented the oncogenic activity of the fusion protein. In further support of the functional importance of the PHD finger, fusion of NUP98 to other PHD fingers that recognize H3K4me3 resulted in an analogous oncogenic phenotype. Microarray analysis revealed a set of developmentally important transcription factors that were upregulated by the fusion protein, thus implicating this transcriptional program in blocking hematopoietic differentiation and inducing AML. Collectively, these findings provide new insights into how PHD finger deregulation induces leukemia. (*Nature*, published online 10 May 2009, doi:10.1038/nature08036) *JK*