RNA MODIFICATION

Reading Sex-lethal

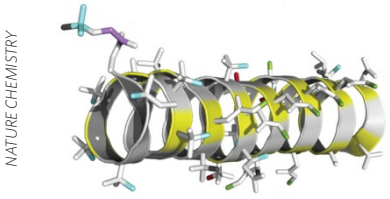
Nature **540**, 242–247 (2016)
Nature **540**, 301–304 (2016)

N^6-Methyladenosine (m^6A) RNA modification is mediated by components of a methyltransferase complex (including METTL3, METTL14 and WTAP) and is subsequently recognized by YTH domain RNA-binding proteins. METTL3 depletion in mice and plants results in embryonic lethality that precludes elucidation of additional functions of m^6A modifications past embryogenesis. Lence *et al.* and Haussmann *et al.* characterized the *Drosophila* METTL3 (Ime4) and METTl14 (dMettl14) homologs and revealed a role for m^6A in regulating sex determination and dosage compensation. Although these mutant flies were viable, there was a significant decrease in female mutants compared to males due to impaired dosage compensation in females. Transcriptome analysis of *Drosophila ime4* mutants exhibited alterations in alternative splicing of *Sex-lethal* (*sxl*), the master regulator of female sex determination and dosage compensation. Alternative splicing of *Sxl* ensures production of functional Sxl protein in females only. Surviving adult female flies that were deficient in *Ime4* exhibited defects in female-specific *Sxl* alternative splicing that resulted in sexual transformations. m^6A directly modulates *Sxl* splicing as *Sxl* mRNA is m^6A methylated and the m^6A reader YTH–521-B was found to bind to a region near the regulated exon within the flanking *Sxl* intron. Overall, these findings reveal an important role of m^6A in regulating sex determination and dosage compensation. *GM*

BIOSYNTHESIS

Terrifically tailored peptides

Nat. Chem. doi:10.1038/nchem.2666 (2016)



Polytheonamides are ribosomally synthesized and post-translationally modified peptide (RiPP) natural products made by a bacterial symbiont in a marine sponge. Among other modifications, the peptide features alternating L- and D-configured amino acids, likely causing it to adopt a β-helical structure. Using heterologous expression in *Escherichia coli* and *in vitro* reconstitution with select precursor peptide variants, Freeman *et al.* characterized the set of enzymes that comprise the polytheonamide biosynthetic pathway and found that only seven enzymes are sufficient to install dozens of post-translational modifications. A single epimerase iteratively introduces all 18 D-amino acids in a C- to N-terminal direction, and then an *N*-methyltransferase installs methyl groups on D-asparagine residues. The biosynthetic pathway also involves β-hydroxylation of valine and asparagine residues by an oxygenase, modification of the N-terminal residue of the core peptide by a dehydratase and removal of the leader peptide by a protease. To reconstitute the 17 methylations that occur at unactivated carbon centers, the authors also used an unconventional rhizobial heterologous host for expression of the two cobalamin-dependent *C*-methyltransferases, each of which act on distinct halves of the core peptide. This detailed understanding of polytheonamide biosynthesis adds new options to the collective toolbox of RiPP biosynthetic enzymes, which could be used to engineer new potentially bioactive compounds. *CD*

PANCREATIC DEVELOPMENT

Changing identity

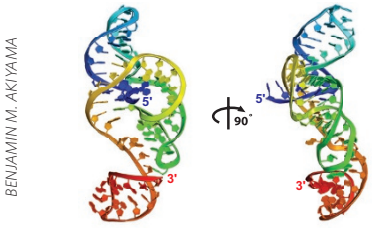
Cell doi:10.1016/j.cell.2016.11.010 (2016)

Cellular plasticity can be induced in pancreatic cell types and results in trans-differentiation. Forced overexpression of the transcription factor Pax4 is sufficient to convert alpha (α) cells into beta (β) cells, while overexpression of the transcription factor Arx does the opposite to reduce insulin production. Li *et al.* performed a chemical screen to identify small molecules that could reverse Arx-mediated conversion of β cells into α cells and enable production of insulin. Their leading candidate was artemether, a known compound that is used to treat malaria but has no defined cellular target, as a promoter of β-cell identity due to inhibition of Arx nuclear localization. Chemoproteomic analysis using cellular pulldowns and MS analysis revealed that artemether directly interacted with gephyrin and promoted its stability. Gephryin, which is known to transport $GABA_A$ receptors to the membrane, stimulated $GABA_A$-receptor-mediated cellular Cl influx that enabled blocking of glucagon secretion in α cells, which resulted in the loss of α-cell identity. Artemether treatment in a zebrafish β-cell ablation model, wild-type mice and human islets increased pancreatic β-cell size and number and upregulated insulin expression and secretion in α cells. Future studies will need to mechanistically connect the upregulation of GABA signaling with the loss of ARX nuclear localization. *GM*

RNA STRUCTURE

Untying Zika's knots

Science **354**, 1148–1152 (2016)



Recent outbreaks of Zika virus (ZIKV), associated with neuronal disorders and birth defects in humans, have focused research into ZIKV biology as a means to identify targets for therapeutic intervention. Studies in related flaviviruses (such as West Nile virus and Dengue virus) have demonstrated that degradation of viral single-stranded RNA genomes by the human exonuclease Xrn1 generates subgenomic flaviviral RNAs (sfRNAs) in infected cells. Akiyama *et al.* now demonstrate that sfRNAs are also generated during ZIKV infection in a process involving steric blockage of Xrn1 processing by a structured RNA motif. Northern blotting analysis of ZIKV-infected mosquito, monkey and human cell lines and *in vitro* Xrn1 digestion assays revealed two major sfRNA fragments that correlated in size with two predicted Xrn1-resistant RNA (xrRNA) elements in the 3′ UTR of ZIKV genomic RNA. X-ray crystallographic analysis of the upstream xrRNA1 element revealed a unique fold that includes a three-way junction, a central pseudoknot and a conserved 14-nucleotide ring motif that lassoes the 5′ terminal strand. While mutations that disrupt base pairing in the ring motif have limited effects on sfRNA production, perturbation of the pseudoknot structure enhances Xrn1 processing and reduces sfRNA abundance in infected cells. Docking studies suggest a tight association of folded xrRNA1 with Xrn1, offering a potential RNA–protein interaction that could be a target for small-molecule or vaccine development. *TLS*

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