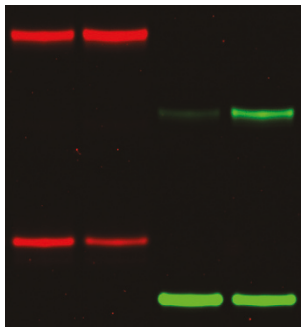
Ribozyme crosses chirality borders



Ribozymes are RNA molecules that can catalyze biochemical reactions including polymerization of other RNA molecules. Their discovery provided support to the 'RNA world' hypothesis, which postulates that life based on RNA might have been the first to emerge on Earth, given RNA's ability to store information like DNA and to catalyze biochemical reactions like protein enzymes. However, all polymerization reactions by ribozymes observed so far occur only in a homochiral system, and the nonenzymatic polymerization of RNA is halted by the incorporation of ribonucleotides of the opposite handedness. This enantiomeric cross-chiral inhibition created a quandary because both D- and L-RNA probably coexisted in prebiotic Earth, before D-RNA affirmed itself as the dominant enantiomer. Hence, it has remained puzzling how ribozymes could have overcome the chiral inhibition in the presence of both enantiomers as substrates. Now, Sczepanski and Joyce show that polymerization of both D- and L-RNA may have emerged together, thanks to ribozymes capable of cross-chiral RNA polymerization. By using *in vitro* evolution, they generated an 83-nucleotide D-ribozyme that could catalyze ligation of L-RNA mono- or oligonucleotides on an L-RNA template. Similarly, the mirror-image L-ribozyme was able to catalyze the reaction of D-enantiomers. Both D- and L-ribozymes were not subject to cross-chiral inhibition, because they could perform the catalysis in the presence of a mixture of substrates and templates of both handed versions. The cross-chiral ribozyme catalyzed the ligation a million times faster than the noncatalyzed reaction, recognized the substrates on the basis of tertiary, sequence-independent contacts and joined substrates regardless of their length, provided that they were bound to a complementary template. Notably, the D-ribozyme could assemble L-oligonucleotides into a fully functional mirror copy of itself, also capable of cross-chiral catalysis. Why homochiral ribozymes eventually emerged remains an open question, and the authors speculate that an achiral polymer able to catalyze both D- and L-RNA might have preceded RNA-based life. (*Nature* **515**, 440–442, 2014) *CD*

Lnc-ing BCAR4 to metastasis

The breast cancer antiestrogen resistance 4 (*BCAR4*) gene is an inducer of tamoxifen resistance in tumor cells, and its expression correlates with cancer aggressiveness. Yang and colleagues now show that the BCAR4 transcript is a lncRNA that promotes metastasis by activating the expression of a cell-migration pathway. lncRNA array screens and quantitative RNA analyses revealed that BCAR4 was the most highly upregulated lncRNA in breast cancer tissues, and its expression correlated with metastasis in multiple cancer cell types. BCAR4 knockdown in breast cancer cell lines specifically reduced expression of genes controlled by the Hedgehog signaling pathway, whose regulatory proteins include the kinase citron (CIT) and transcription factors GLI2, PNUTS and SNIP1. PNUTS and SNIP1 directly bind distinct regions of BCAR4 in RNA pulldown assays, whereas CIT and GLI2 interact with the lncRNA via SNIP1. CIT phosphorylates GLI2 at Ser149, and this phospho-GLI2 form is enriched within the BCAR4 complex and in invasive breast cancer tissues. A screen of chemokines known to activate CIT kinase in breast cancer cell lines revealed that CCL21, previously implicated in breast cancer metastasis, induced CIT-dependent GLI2 phosphorylation and nuclear translocation. In the nucleus, GLI2 directs expression of genes involved in cell migration. Indeed, phospho-GLI2 and BCAR4 bind CCL21-induced genes in ChIP assays and are required for their expression. Knockdown of BCAR4 or its associated proteins inhibits cell migration, thus supporting the central role of this lncRNA in the signaling pathway. Moreover, BCAR4-SNIP1 binding precludes SNIP1 interactions with p300, which would repress the acetyltransferase activity of the latter. This leads to increased histone acetylation at CCL21-induced genes. Intriguingly, the authors show that acetylated histones are recognized by PNUTS, which inhibits PP1 phosphatase activity at the RNA Pol II C-terminal domain, thus suggesting a potential means for BCAR4 to regulate transcription. The current findings, together with the demonstration that BCAR4 can be efficiently depleted with locked nucleic acid (LNA) antisense oligonucleotides, underscore the potential of new cancer therapies that target lncRNAs. (*Cell* **159**, 1110–1125, 2014) *BM*

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A CRISPR cut to bacteria

Broad-spectrum drugs that treat infections also destroy host-beneficial microbial communities, and long-term antibiotic use has led to the emergence of multidrug-resistant opportunistic bacterial pathogens, such as carbapenem-resistant Enterobacteriaceae and methicillin-resistant *Staphylococcus aureus* (MRSA).

Two independent groups have now developed a new class of antimicrobials that act on specific bacterial populations, while leaving others unharmed. These new antimicrobials are based on the *Streptococcus pyogenes* type II CRISPR gene-editing system, which directs the Cas9 nuclease to cleave genomic target sites that can be specified in CRISPR guide RNAs (crRNAs). Lu and colleagues targeted enterobacterial genes encoding β-lactamase enzymes that conferred extended-spectrum or pan–β-lactam antibiotic resistance, whereas Bikard, Marraffini and colleagues studied the specific elimination of kanamycin-resistant or MRSA cells. Both groups showed that transforming bacteria with plasmids bearing Cas9 and crRNAs that targeted specific antibiotic-resistance factors was able to promote killing of the intended bacterial populations without affecting cells that were not carrying the targeted sequences. Lu and colleagues also demonstrated that Cas9–crRNA modules could be introduced into target bacterial cells through conjugation with engineered donor bacteria containing mobilizable plasmids or by infection with M13 phagemids. The latter approach was used to modulate the composition of a complex microbial community *in vitro* and was also efficient in treating *Escherichia coli* O157:H7 infection in an insect larva model. Bikard, Marraffini and colleagues used a phagemid-based approach to target kanamycin-resistant *S. aureus* mixed with kanamycin-sensitive bacteria and found that the nontargeted cells outcompeted any residual targeted cells for growth. The group also showed that a single crRNA construct could successfully be programmed against two separate virulence plasmids in an MRSA strain. Phagemid treatment of antibiotic-sensitive *S. aureus* could immunize the cells against the transfer of antibiotic-resistance genes from infection with phage grown on the MRSA strain. Selective targeting of kanamycin-resistant bacteria was also demonstrated in a mouse skin colonization model. Notably, both groups found that Cas9-targeted escapees that arose after treatment were due to defects in the CRISPR constructs rather than to host-adaptive mutations that created resistance to the new drug, thus supporting the concept of CRISPR-based treatments as an alternative to traditional drug therapies. (*Nat. Biotechnol.* **32**, 1141–1145, 2014 and *Nat. Biotechnol.* **32**, 1146–1150, 2014) *SG*