enacted at one place cannot have an effect at another place unless there would have been time for a light signal to get from the first place to the second place. The speed of light is relevant because, according to Einstein's theory of relativity, no causal influence can travel faster than light.

A Bell inequality is a mathematical relationship regarding the statistics of measurement outcomes obtained by two or more parties, and also involving the measurement settings chosen by those parties. Suppose that the parties are in well-separated laboratories, and that the measurement settings are chosen and implemented, and the outcomes obtained, in a sufficiently short time that the only way the choice of setting by any party could affect the outcome of any other party would be through a faster-than-light influence. Then, by definition, all Bell inequalities will be satisfied by all local-realistic theories. An experiment violating a Bell inequality therefore implies that either locality or realism is false. Bell's theorem is that, according to quantum mechanics, such an experiment is possible if the parties share particles prepared in a suitable entangled state. Entanglement is a holistic property of a system of quantum particles that can persist even when the particles are far apart.

Bell inequalities have been violated experimentally many times before[4–9]. However, all of these experiments had loopholes. Either the parties were not far enough apart, given how long it took for the processes involved (randomly choosing a setting, adjusting the apparatus appropriately and obtaining an outcome), or the measurements were inefficient, so that quite often no outcome at all was registered. The inefficiency is relevant because it can allow the existence of local realistic theories — albeit highly contrived ones — that exploit the existence of null outcomes to simulate the correlations of quantum mechanics.

Several groups worldwide have been racing to perform the first Bell experiment that combines large separation, efficient detection and fast operation of the apparatus. Hensen *et al.* have won the race by using a new scheme. Previously, the leading approach was to prepare an entangled state of two photons, send one to one laboratory — conventionally called Alice's — and the other to a second laboratory, Bob's. By contrast, Hensen and colleagues' experiment should be regarded as using a three-party Bell inequality.

In this three-party approach (Fig. 1), Alice and Bob each prepare an entangled state of a photon and an electron, keep their electrons in a diamond lattice and send their photons to Juanita, as I'll call her. Alice and Bob then each choose a setting and measure their electrons, which can be done efficiently, while Juanita performs a joint measurement on the two photons. Alice's and Bob's outcomes will be purely random unless Juanita gets a rare 'successful' result, in which case the outcomes indicate entanglement between Alice's and Bob's electrons. Unlike Alice and Bob, Juanita always makes the same measurement, and so its inefficiency does not open a loophole.

Hensen and co-workers' experiment was made possible only by combining state-of-the art quantum technologies — it was performed in the Netherlands, but used diamond substrates prepared in the United Kingdom and fast random-number generators developed in Spain. Maintaining optimal operation of all the devices during the experiments was extremely challenging, and the rate of events (defined as Juanita getting a successful outcome) was only about one per hour. As a consequence, only 245 such events were recorded, and the statistical uncertainty in the reported Bell-inequality violation is comparatively large. Nevertheless, from a careful analysis of the entire data set, including runs in which Juanita did not get the desired outcome, Hensen *et al.* reject the local-realism null hypothesis at a confidence level conventionally considered to be statistically significant. It is to be hoped that more data will be generated soon.

The authors' approach might allow them to implement quantum-information protocols enabling secure communication, even when the devices used are not trusted by the users. For this to be practical, the event rate would have to be massively increased above its current level. However, the basic technology and the scheme (involving joint measurements by the intermediary Juanita) are promising.

The immediate significance of the reported experiment, however, is in hammering the final nail in the coffin of local realism. Some almost metaphysical loopholes remain open — if the results can be replicated with humans, rather than machines, freely choosing the measurement settings and consciously registering the outcomes, then the coffin will have been interred and buried. That experiment, however, is for many years hence. For the moment, we should celebrate Hensen and colleagues' landmark achievement in physics. ■

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NON-CODING RNA

Antibiotic tricks a switch

A screen for compounds that block a bacterial biosynthetic pathway has uncovered an antibiotic lead that shuts off pathogen growth by targeting a molecular switch in a regulatory RNA structure. SEE ARTICLE P.672

THOMAS HERMANN

The golden age of antibiotic discovery, between 1940 and 1960, was heralded by the work of Selman Waksman. A biochemist and microbiologist, Waksman coined the term 'antibiotic' and was the first to use systematic screening to discover antibacterial leads[1]. Efforts in his laboratory yielded more than 20 natural antibiotics — most notably streptomycin in 1943, the first effective treatment for tuberculosis[2]. The discovery won Waksman the 1952 Nobel Prize in Physiology or Medicine. Waksman's research caught the attention of scientists at the US pharmaceutical company Merck, and the ensuing collaboration was instrumental in developing streptomycin for clinical use. On page 672 of this issue, Howe *et al.*[3] from the research laboratories of the present Merck describe a new antibiotic lead, identified using a sophisticated refinement of the phenotypic-screening approach introduced by Waksman.

Seven decades after Waksman's research, the flood of antibiotics emerging from natural sources has dwindled to a trickle, and, for various reasons[4], few companies remain active in antibiotic drug discovery. This is despite an urgent clinical need for new agents in the face of rising resistance to existing antibiotics[5]. On this background, Howe and colleagues' surprising discovery of a drug target in a bacterial non-coding RNA (ncRNA) provides a welcome bright spot.

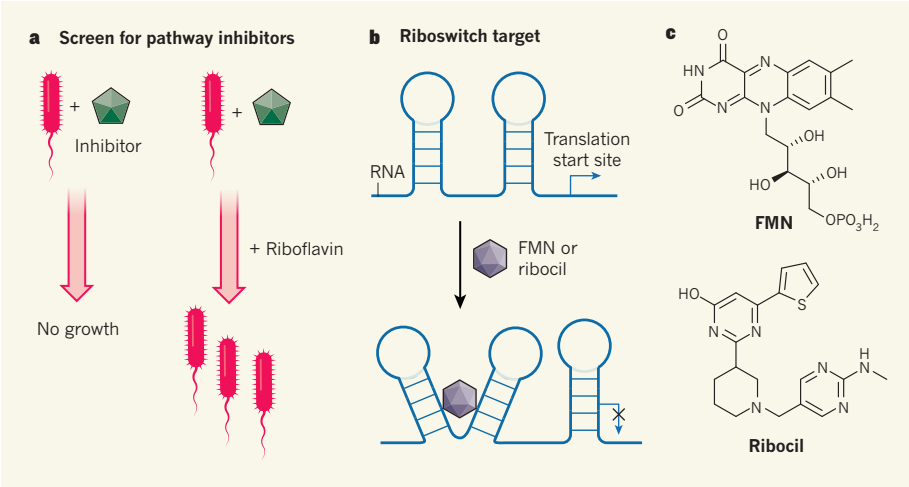

Figure 1 | Metabolic-pathway blockade identifies an antibiotic. **a**, Bacteria use a metabolic pathway to synthesize riboflavin — a molecule involved in many enzymatic reactions — when environmental riboflavin is not available. Howe *et al.*[3] searched for inhibitors of this pathway by identifying compounds whose antibacterial action could be attenuated by riboflavin addition. **b**, The screen uncovered the synthetic molecule ribocil as a selective inhibitor of riboflavin biosynthesis. The authors report that ribocil binds to a 'riboswitch' regulatory domain in a non-coding region of the messenger RNA that encodes a synthase enzyme involved in riboflavin synthesis. The riboswitch is normally bound by flavin mononucleotide (FMN), a metabolite produced from riboflavin; such binding induces a structural change in the riboswitch that prevents expression of the synthase enzyme and thus stops further riboflavin synthesis when sufficient FMN is available. **c**, Ribocil, despite having a very different structure from FMN, also binds at this site, tricking the switch into shutting off riboflavin production and depriving the bacterium of this essential metabolite.

Phenotypic screening involves applying test compounds to bacterial cultures and observing changes to the cells' characteristics (their phenotype). Since Waksman's days, the simplest phenotypic screen for antibacterial activity has been to look for growth inhibition — cell death is the most severe of phenotypes and is readily measured. Howe and colleagues established a more subtle screen that interrogates a single bacterial metabolic pathway. Unlike humans, bacteria can synthesize the metabolite riboflavin, also called vitamin B2, which is a precursor of the cofactors required in many enzymatic reactions. The most prominent of these cofactors is flavin mononucleotide (FMN). Genes involved in riboflavin biosynthesis are essential only when the bacteria cannot acquire the vitamin from its environment. The researchers hypothesized that antibacterial compounds whose growth-suppressing effect could be reversed by supplementing the bacterial cultures with riboflavin would be candidate inhibitors of the riboflavin biosynthesis pathway (Fig. 1a). They tested around 57,000 synthetic small molecules and identified the molecule ribocil as one that kills bacteria by selectively blocking riboflavin biosynthesis.

To confirm ribocil's mechanism of action, Howe and colleagues demonstrated that bacteria treated with the compound were indeed depleted in riboflavin. In mice infected with pathogenic bacteria, ribocil treatment reduced the concentration of bacteria by more than 1,000-fold, which is a promising start. The team went on to identify the molecular target of ribocil by isolating bacterial clones that became resistant during prolonged exposure to ribocil at sublethal concentrations, and sequencing their genomes. What initially seemed a routine exercise turned to excitement when the resistant bacteria were found to harbour mutations in a non-coding stretch of the bacterial genome, suggesting that ribocil blocks a mechanism of gene regulation rather than inhibiting a protein target. Further sleuthing for ribocil's site of attack led the researchers to a highly structured ncRNA domain upstream of the sequence that marks the translational start site in a messenger RNA encoding a key synthase enzyme in the riboflavin biosynthesis pathway.

It turns out that the ncRNA domain bound by ribocil is normally bound by FMN, and the site is a 'riboswitch', the term used for RNA regions that change structure when bound by a ligand (Fig. 1b). Binding of FMN traps the riboswitch RNA in a configuration that prevents access of transcription and translation machinery and thus blocks expression of the synthase enzyme. This mechanism provides a way for bacteria to reduce further production of riboflavin, and thus FMN, when sufficient amounts of the cofactor are available. By binding to the same ncRNA target, ribocil tricks the riboswitch to respond, shutting off riboflavin production and depriving the bacteria of the essential metabolite. As a compelling piece of evidence, the researchers used X-ray crystallography to provide a snapshot of ribocil in the act of binding to the riboswitch RNA. Such structural information will be valuable for improving the antibacterial activity of ribocil derivatives for potential clinical use.

This mechanism of mimicking a natural ligand of a riboswitch is not known from any other antibacterial compounds. The study thus conclusively demonstrates the advantages of phenotypic screening that allows all constituent components of a pathway — proteins and nucleic acids — to be tested simultaneously in an unbiased fashion. Although riboswitches are obvious targets for antibacterial discovery[6], Howe and colleagues have for the first time identified a riboswitch-binding molecule that is not a close structural analogue of a metabolite ligand. This improves the odds that ribocil will have no off-target effects on other pathways that involve riboflavin and FMN, which is already hinted at by the authors' observation that even high doses of the compound were not toxic in mice.

From a broader perspective, Howe and colleagues' research is a striking demonstration that structured regions in ncRNA may serve as targets for synthetic drugs. There are only a few previous instances of ncRNA elements being exploited as potential drug targets, one being a region in the hepatitis C virus that is bound by synthetic inhibitors of viral protein synthesis[7]. But many natural antibiotics[8] work by interfering with protein synthesis through targeting ncRNA components in the bacterial ribosome, the cellular machine that synthesizes all proteins.

Assuming that nature is on to something by targeting ncRNA in the ribosome, an optimist might point out that the plethora of ncRNAs recently discovered in the genomes of bacteria and more-complex organisms will provide a rich expansion of the range of targets for therapeutic intervention[9,10]. For the sceptics, Howe and colleagues' work might allay doubts that targeting ncRNA elements may deliver new drugs. And we can hope that clinicians who urgently need alternative antibiotics will be among the first to reap the benefits of RNA emerging as a drug target. ■

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