

**Figure 1** Cheaters and secretors. Cooperator *Pseudomonas aeruginosa* secrete a green siderophore, pyoverdine, which makes iron available for uptake by all cells. Cheaters save on the expense of producing pyoverdine but can exploit the siderophores of nearby secretors. Griffin *et al.*<sup>5</sup> tested how the proportion of each varied over time in response to relatedness (high or low) and scale of competition (global or local). As these results show, relatedness is crucial but it is modulated by the scale of competition. (Simplified from ref. 5.)

confirming result: the local-competition treatments yielded more cheaters than the global-competition ones. Kinship is crucial, but it is modulated by the scale of competition. Local competition exactly cancelled out the effect of high relatedness, such that neither type was favoured (Fig. 1).

There is an interesting history behind the theories tested here. The first study to challenge Hamilton's ideas about limited dispersal was a computer simulation based on group-selection theory<sup>2</sup>. This theory divides selection into within-group and between-group components, with the latter favouring cooperation. Group selection has a shady past. At one time, some biologists saw group-selected cooperation everywhere in nature, even though the process was unsupported by rigorous models, and consequently the whole idea was brought into disrepute. But there has been growing agreement that group selection, properly applied, is a roughly equivalent way of understanding social selection — not so much an alternative theory as an alternative means of slicing up the way in which selection occurs. Thus, kin-selection theorists took the computer-simulation results<sup>2</sup> seriously and quickly found two different ways of squaring them with their theory. First, one can add in the indirect effects for the cases where helping a neighbour takes away fitness from another, perhaps equally related, neighbour<sup>3</sup>. Alternatively, relatedness can be rescaled so that it measures genetic similarity

relative to the locally competing individuals, rather than to the global population<sup>4</sup>.

It is the relativistic effect of kinship that Griffin *et al.* set out to show, and they have succeeded. Curiously, however, their experiment is perhaps more easily understood from a group-selection standpoint. The conditions of low and high relatedness correspond exactly to the presence and absence of within-group selection. The conditions of global and local competition correspond exactly to the presence and absence of between-group selection. The two-by-two crossing of these treatments therefore leads to the most basic group-selection experiment possible. The results confirm that cooperation is favoured by between-group selection and disfavoured by within-group selection.

Does all this mean that we should discard kin selection in favour of the simpler group-selection approach? Hardly. Kin selection has yielded far more insights into the complex behaviours of animals such as social insects. For example, elegant theories of sex-ratio conflict in social insects emerge naturally from kin-selection models, whereas the corresponding group-selection models

are so complex that they have not been developed. But this history does suggest that group selection has matured enough for it to become a partner with kin selection in contributing insights, though the story is not without some irony. Once, group selectionists saw cooperation everywhere but were brought down to earth by individual selectionists. Now group selection is being used, not to show the ubiquity of cooperation but to rein in theories on an important form of cooperation envisaged by individual selectionists.

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## Chemical biology

# Green fluorescent RNA

Michael Famulok

The future for intracellular imaging looks bright with the development of fluorescent probes made entirely of RNA. The cunning design exploits structural attributes of RNA to detect a variety of small molecules.

Green fluorescent protein (GFP) has proven tremendously useful as a method of creating intense visible fluorescence by entirely molecular biological means<sup>1</sup>. Generally, the gene encoding GFP is fused with a gene of interest so that the resultant protein is tagged with the fluorescent module. It can then be followed in real time by optical imaging, to decipher spatio-temporal information on gene expression or protein localization within living cells or tissues<sup>2</sup>. Such information is crucial for understanding complex cellular processes that depend on when, where and how much of a protein is present. The challenge now is to devise similar methods to reveal other molecules involved in cellular functions, such as small-molecule messengers, drugs, metabolites and short functional RNAs. Stojanovic and Kolpashchikov<sup>3</sup>, writing in the *Journal of the American Chemical Society*, report a considerable advance in this area with the development of RNA-based probes that can detect small organic molecules by fluorescence.

The new concept relies on aptamers — short RNA sequences that can bind specifi-

cally to particular ligands<sup>4</sup>. These molecules are fairly recent discoveries, but already they have been adapted to make probes for various molecules<sup>5</sup>. However, current aptamer probes are generated and labelled with fluorescence or radioactivity outside the cell, and a delivery mechanism is needed to get them inside — a procedure that is potentially disruptive to the very processes under observation. Stojanovic and Kolpashchikov have sidestepped this problem by creating an aptamer probe that can be genetically encoded and produced inside the cell, and that is able to generate marked fluorescence by itself.

The basis of their probe is an aptamer that can bind to the dye malachite green<sup>6</sup> (MG). The dye itself is not fluorescent, but its structure is such that when it binds to the aptamer it is forced into a rigid conformation that is highly fluorescent<sup>7</sup>. This, however, is only half the story. Stojanovic and Kolpashchikov's probe has a second module — another aptamer that binds to whatever small molecule is to be detected.

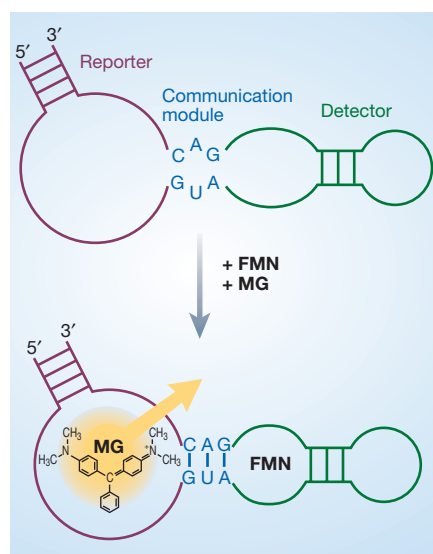
To form the final probe, the authors take advantage of an intrinsic property of

aptamers: adaptive binding of ligands. Ever since the first nuclear magnetic resonance structures of aptamer–ligand complexes, it has been clear that the binding of a ligand by an aptamer occurs almost exclusively by adaptive recognition<sup>4</sup>. For example, aptamers often contain unpaired loop or bulge regions, which are disordered in the free nucleic acid and only acquire a defined conformation by adaptive folding around the ligand.

In the new probe, the MG aptamer and the detector aptamer are linked by a communication module. This module substitutes for one of the helices in the MG-aptamer domain, the formation of which is absolutely required for binding to the dye. When a ligand binds to the detector unit, adaptive binding forces the communication module into a helical structure, providing the right conformation for the MG aptamer to bind to the dye. The function of the short communication element is thus to render the disparate RNA domains interdependent, such that the probe can bind only to MG — and hence generate fluorescence — once a ligand has bound to the detection module (Fig. 1). It also means that the final sensor probe consists entirely of RNA and can therefore be generated by genetic encoding, requiring only the exogenous addition of the dye molecule to the growth medium to become an intracellular sensor.

In this respect, the aptamer probes are actually more analogous to another important genetic reporter system — firefly luciferase — than to the autofluorescent GFP. Luciferase requires the addition of a small-molecule substrate, luciferin, to function as a light-emitting reporter for the presence of a protein. The beauty of the new aptamer system is that it promises to have many of the advantages of GFP and luciferase, particularly in that the cells under investigation should be perturbed only minimally. The probes are less bulky than either GFP or luciferase, and could be designed for expression in cells without requiring covalent attachment to any protein, unlike GFP or luciferase. In addition, their modular design could potentially be useful for the intracellular detection of a large variety of molecules.

So far, however, the new aptamer probes are merely a proof of principle. To permit widespread cellular application, they still require optimization and improvement with regard to more straightforward design, choice of the chromophore, and sensitivity. Stojanovic and Kolpashchikov's first set of probes use known aptamers that bind to adenosine triphosphate<sup>8</sup>, theophylline<sup>9</sup> or flavin mononucleotide<sup>10</sup> to detect the molecules *in vitro*. Reporters that can trace compounds such as these are highly desirable because small-molecule messengers, such as Ca<sup>2+</sup> ions, cyclic nucleotide monophosphates and lipids, often act locally and



**Figure 1** Fluorescent RNA-based probes. The probes designed by Stojanovic and Kolpashchikov<sup>3</sup> consist of a reporter module that binds to the dye malachite green (MG), a communication module and a detector module, which in this example binds to flavin mononucleotide (FMN). The binding of FMN to the detector unit induces helix formation in the communication module. This helix completes the structure required to bind to the dye, which fluoresces only once it is bound to the probe.

transiently to control cellular functions, and systems to follow them are scarce<sup>2</sup>.

It is possible that the MG-aptamer module could also be developed for the visualization of functional nucleic acids. Indeed, the fact that it is entirely based on nucleic acid would seem to predestine it for that purpose. Cells can silence certain genes at the stage

of translation by a mechanism that involves short single-stranded RNAs known as miRNAs. This process allows protein expression levels to be altered very quickly<sup>11</sup>. The detection of miRNAs in live cells and tissues will help to address questions about their genetics, biogenesis, trafficking and function. Progress is already being made towards this end: recent work from my group used a design based on an RNA enzyme to develop fluorescent probes for specific miRNAs<sup>12</sup>.

Together these studies highlight the importance of developing versatile methods for revealing the myriad cellular molecules as they move and change over time. Future endeavours in this area promise to revolutionize our insight into the fascinating interplay between the compounds that keep cells up and running.

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### Transmissible spongiform encephalopathies

## Prion proof in progress

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Whether a protein can transmit disease in mammals has been an open question for some time. The latest test of this idea provides some strong evidence in favour, but is unlikely to end the debate.

**S**tudies of the infectious agent responsible for the transmissible spongiform encephalopathies have spanned more than six decades. But the slow course of these diseases, and experimental difficulties in their study, has left the nature of this agent in doubt. Now, a report in *Science* by Legname *et al.*<sup>1</sup> represents a major step towards proving that a protein is the only essential element of the infectious agent.

Transmissible spongiform encephalopathies (TSEs) are uniformly fatal neurodegenerative diseases, and include scrapie in sheep, Creutzfeldt–Jakob disease (CJD) in humans, bovine spongiform encephalopathy

(BSE) and the latter condition transmitted to humans (variant CJD). The discovery that the infectious agent that causes scrapie is far more radiation-resistant than known viruses or bacteria — which rely on the very radiation-sensitive DNA or RNA — led to the suggestion<sup>2</sup> that this agent is a protein (without a required nucleic acid). Proteins are far less sensitive to radiation damage than are DNA and RNA. With the genetic<sup>3</sup> and biochemical<sup>4–7</sup> identification of PrP, a protein essential for the propagation and infectivity of TSEs<sup>8</sup>, the ‘protein-only’ hypothesis<sup>2,9</sup> took concrete form (‘prion’ means ‘infectious protein’).

The protein PrP is a cell-surface protein