Selected Reading

1. O'Hagan. D. (1991). The Polyketide Metabolites (Chichester, UK: Ellis Horwood).
2. Staunton, J., and Weissmann, K.J. (2001). Nat. Prod. Rep. *18*, 380–416.
3. Hopwood, D.A. (1997). Chem. Rev. *97*, 2465–2497.
4. Shen, B. (2003). Curr. Opin. Chem. Biol. *7*, 285–295.
5. Moore, B.S., and Hertweck, C. (2002). Nat. Prod. Rep. *19*, 70–99.
6. Bililign, T., Hyun, C.-G., Williams, J.S., Czisny, A.M., and Thorson, J.S. (2004). Chem. Biol. *11*, this issue, 959–969.
7. Maeda, K., Takeuchi, T., Nitta, K., Yagishita, K., Utahara, R., Osato, T., Ueda, M., Kondo, S., Okami, Y., and Umezawa, H. (1956). J. Antibiot. (Tokyo) *9*, 75–81.
8. Hansen, M.R., and Hurley, L.H. (1996). Acc. Chem. Res. *29*, 249–258.
9. Wilkinson, B., Foster, G., Rudd, B.A., Taylor, N.L., Blackaby, A.P., Sidebottom, P.J., Cooper, D.J., Dawson, M.J., Buss, A.D., Gaisser, S., et al. (2000). Chem. Biol. *7*, 111–117.
10. Hardt, I.H., Steinmetz, H., Gerth, K., Sasse, F., Reichenbach, H., and Höfle, G. (2001). J. Nat. Prod. *64*, 847–856.
11. Gaitatzis, N., Silakowski, B., Kunze, B., Nordsiek, G., Blöcker, H., Höfle, G., and Müller, R. (2002). J. Biol. Chem. *277*, 13082–13090.
12. Mochizuki, S., Hiratsu, K., Suwa, M., Ishii, T., Sugino, F., Yamada, K., and Kinashi, H. (2003). Mol. Microbiol. *48*, 1501–1510.
13. Olano, C., Wilkinson, B., Moss, S.J., Braña, A.F., Méndez, C., Leadlay, P.F., and Salas, J.A. (2003). Chem. Commun. (Camb.) *21*, 2780–2782.
14. He, J., and Hertweck, C. (2003). Chem. Biol. *10*, 1225–1232.
15. Olano, C., Wilkinson, B., Sanchez, C., Moss, S.J., Sheridan, R., Math, V., Weston, A.J., Braña, A.F., Martin, C.J., Oliynyk, M., et al. (2004). Chem. Biol. *11*, 87–97.

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Ribozyme Diagnostics Comes of Age

Biosensing ribozymes could soon be used to diagnose viral infection. The Kossen group from Sirna Therapeutics have developed a sensitive, high-throughput means of screening for hepatitis C virus, using their target activated half-ribozyme technology, as reported in the June issue of *Chemistry & Biology* [1].

A little over two decades ago, the first demonstrations of catalytic RNAs (ribozymes) were reported in the literature [2, 3]. These ribozymes were the cleavage-ligation catalyzing Tetrahymena ribosomal RNA intron [3] and the RNA component of the endoribonuclease RNase P, which catalyzes the site-specific cleavage of the 5′ precursor segment of transfer RNAs [2]. Subsequent to these important findings, several other smaller, naturally occurring ribozymes with RNA cleavage and ligation activities have been described [4], and most recently the RNA component of the large ribosomal subunit has been identified as the ribosomal transpeptidase [5]. The spectrum of the catalytic capabilities of RNA was greatly enhanced following the development of techniques for in vitro evolution of RNAs with new capabilities ranging from ATP hydrolysis to polymer biosynthesis [6]. An important development for evolving biologically and chemically useful ribozyme functions is the addition of allosteric activation functions to ribozymes, which can be mediated by binding of a variety of ligands ranging from small organic molecules through proteins or nucleic acid oligomers [7, 8]. Allosteric activation is the key to generating biosensing nucleic acids that can be used to monitor any of a number of biological or chemical processes. Ribo-reporters could in essence become inexpensive replacements for antibodies and other methods currently in use for diagnostic testing.

Last year, a group from Sirna Therapeutics reported the development of a target activated ribozyme capable of detecting zeptomole (10^{-21}M) quantities of hepatitis C viral RNA in solution [9]. The key to such sensitive detection properties is that the ribozyme component parts have absolutely no activity in the absence of the cognate substrate, which differs from previously published allosteric ribozymes whose rates of activity are dictated by the rate differences of ribozyme function in the absence and presence of substrate or allosteric modifier. The Sirna group developed a "half-ribozyme" approach in which the target RNA itself serves to complete the ribozyme structure. In this ribozyme reaction scheme, the multiple turnover class 1 ligase motif developed by Bartel and colleagues [10] was truncated to form a bimolecular substrate RNA, which interacts with the target sequence to allow ligation of one of the substrate RNAs containing a 3′ cis-diol with a 5′ triphosphate containing substrate RNA oligo (Figure 1). This half-ribozyme has no ligase activity in the absence of target RNA and displayed an observed rate increase of 4,000,000-fold when bound to the target.

This exquisitely sensitive ribozyme reporter has been further analyzed for detection of HCV sequences and high-throughput assays, as reported in last month's *Chemistry & Biology* by Kossen and coworkers [1]. In this study, naturally occurring variants of HCV that contain mismatched pairings to the half-ribozyme were analyzed for their effects on ribozyme-mediated ligation of the substrate RNAs. By extending the base pairing of the ribozyme to target the mismatched sequences, these were accommodated without significant kinetic impairment of target-dependent ligation rates. The variant HCV sequences represent greater than 80% of the GenBank HCV 5′UTR entries, with one of the sequences representing 66%. Thus, the half-ribozyme assay can be generally applied to the majority of HCV clinical samples.

In order to make this assay suitable for clinical applications, the Sirna investigators collaborated with investigators from Thermo Electron, Corp., Point of Care and

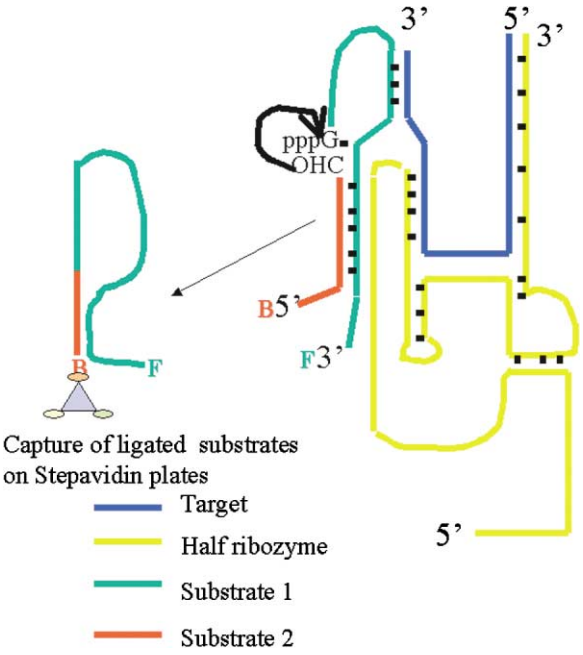

Figure 1. Model of Half-Ribozyme-Mediated Ligation

The half-ribozyme (yellow) binds to the target RNA (blue). Binding of the half-ribozyme to the target results in conformational change that allows substrates 1 (red) and 2 (green) to bind to the half-ribozyme and carry out the indicated ligation step. For high-throughput analyses, the substrates have biotin (B) and fluorescein (F) covalently attached. The ligated oligo is captured on streptavidin-coated plates, and the fluorescence is read or detection is mediated by use of an anti-fluorescein antibody-peroxidase conjugate (see text).

Rapid Diagnostics to develop a high-throughput format for detection of the ligated products. The first step was to modify the substrate oligos for capture and assay in an ELISA-type format. One of the substrate oligos was tagged with biotin on the 3′ end, and the other substrate was modified with a 5′ fluorescein. Thus, ligation of these two oligos generates a product that can be captured on strepavidin-coated microtiter plates. The fluorescein can either be detected directly, or the signal can be amplified by using an alkaline phosphatase-conjugated anti-fluorescein antibody. A second platform for detection of target-dependent ligation of the substrates was an optical immunoassay system in which the ligated probe is captured by an oligo complementary to an extension on the probe. The capture oligo was immobilized on the surface of a silicon wafer. The 5′ biotin on the ligated substrate was detected via interaction with an avidin-peroxidase conjugate. In both assay platforms, the oligo modifications unfortunately compromised the sensitivity of the assay, reducing the target-dependent ligation rate approximately 4-fold, with some increase in the background of spontaneous, ribozyme-independent ligation. Nevertheless, the target-dependent ligation mediated by the half-ribozyme resulted in a target-dependent detection sensitivity several thousand-fold greater than other nucleic acid-activated ribozymes. The sensitivity of the assays using both platforms was about 1 pM, or 3 million molecules of HCV 5′ UTR. This sensitivity is somewhat less than what is generally obtained with immunological assays, which is in the high femtomolar range. The authors attribute the diminished sensitivity obtained using the modified oligo substrates to diminished catalytic efficiency of the half-ribozyme with these substrates relative to unmodified, radiolabeled oligos. Performing additional in vitro evolution experiments with the modified oligos to obtain variants of the half-ribozyme with increased catalytic efficiency when utilizing these substrates can certainly enhance the sensitivities of the high-throughput assays.

Overall, the development of the half-ribozyme as a highly selective and efficient tool for viral RNA diagnostics could have a huge impact on clinical tests for viral infections. The impact will be in both cost of reagents and sensitivity of the assays. Once the high-throughput method for half-ribozyme-mediated ligation can reach the zeptomole level of sensitivity obtained with unmodified oligos, this approach will clearly be superior to currently available immunoassays. The half-ribozyme approach could also eclipse PCR-based assays such as are currently being used for HIV and HCV testing. The ribozyme approach is not only less expensive, but more rapid. Moreover, using the optical chip-based platform, colorimetric readouts should be possible and could be conveniently carried out in a doctor's office. It is heartening to see the great promise of ribozymes once again come to the front, this time in the form of ribozyme-based diagnostics, as opposed to therapeutics.

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Selected Reading

1. Kossen, K., Vaish, N.K., Jadhav, V.R., Pasko, C., Wang, H., Jenison, R., McSwiggen, J.A., Polisky, B., and Seiwert, S.D. (2004). Chem. Biol. *11*, 807–816.
2. Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S. (1983). Cell *35*, 849–857.
3. Kruger, K., Grabowski, P.J., Zaug, A.J., Sands, J., Gottschling, D.E., and Cech, T.R. (1982). Cell *31*, 147–157.
4. Rossi, J.J. (1999). Chem. Biol. *6*, R33–R37.
5. Nissen, P., Hansen, J., Ban, N., Moore, P.B., and Steitz, T.A. (2000). Science *289*, 920–930.
6. Bittker, J.A., Phillips, K.J., and Liu, D.R. (2002). Curr. Opin. Chem. Biol. *6*, 367–374.
7. Silverman, S.K. (2003). RNA *9*, 377–383.
8. Steele, D., Kertsburg, A., and Soukup, G.A. (2003). Am. J. Pharmacogenomics *3*, 131–144.
9. Vaish, N.K., Jadhav, V.R., Kossen, K., Pasko, C., Andrews, L.E., McSwiggen, J.A., Polisky, B., and Seiwert, S.D. (2003). RNA *9*, 1058–1072.
10. Ekland, E.H., Szostak, J.W., and Bartel, D.P. (1995). Science *269*, 364–370.