***in vitro* and *in vivo* association assays.** The GST–cytoplasmic region of EGFR fusion proteins (amino acids 930–1,220, which do not include Lys 721, a key residue in the ATP-binding site) were generated as described[28]. The *in vitro* Jak2 assay was done as before[2], with minimal modification. Proteins immobilized on anti-Jak2 antibody were mixed with 95 μl of 0.1% Triton X-100 reaction buffer[2] containing 10 μM ATP, 0.25 mCi ml^{-1} [γ-^{32}P] ATP and 10 μg GST–EGFR-mutant fusion protein. After reaction, the anti-Jak2 immune complexes were resolved by SDS–PAGE and the autophosphorylation of Jak2 was visualized by autoradiography. To collect GST–EGFR-mutant fusion proteins, the supernatant was incubated with 30 μl glutathione–Sepharose beads; ^{32}P-labelled proteins were then resolved by SDS–PAGE and phosphorylation of GST–EGFR-mutant fusion proteins was visualized by autoradiography. When the *in vitro* association of phosphorylated GST–EGFR-mutant fusion proteins with Grb2 was determined, instead of being visualized by autoradiography, the fusion proteins were incubated with Grb2, processed as described[19], and immunoblotted with anti-Grb2. To determine association of EGFR mutants with Grb2 *in vivo*, and the tyrosine-phosphorylation or MAP-kinase activity in cells expressing EGFR mutants, CHO-GHR cells were transfected with 6 μg of pRc/CMV alone or with WT or EGFR mutant cDNA in pRc/CMV in 6-cm dishes by using the lipofectamine method with modification[29]. Upon cell lysis, precipitated EGFR was immunoblotted with anti-PY (phosphotyrosine), and immunoprecipitates by anti-Grb2 were immunoblotted with anti-EGFR.

Luciferase assay. CHO-GHR cells were plated in 12-well dishes. One day after adenovirus-mediated gene transfer, cells were transfected by using the lipofectin procedure with 0.15 μg fusion gene construct pFOS(-359)Luc[30]. After incubation for 18 h with lipofectin in serum-free Ham's F-12 medium, transfected cells were pretreated for 60 min with or without 30 μM PD98059, then treated with hGH (500 ng ml^{-1}) for 5.5 h. Luciferase was assayed as described[20].

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Peptide bond formation by *in vitro* selected ribozymes

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An attractive solution to the problem of the origin of protein synthesis in an evolving 'RNA world' involves catalysis by nucleic acid without assistance from proteins[1,2]. Indeed, even the modern ribosome has been considered to be fundamentally an RNA machine[3], and the large ribosomal subunit can carry out peptidyl transfer in the absence of most of its protein subunits[4]. Successive cycles of *in vitro* selection and amplification[5–7] have been used to find RNAs that perform many biochemical reactions[8–16], including transfer of an RNA-linked amino acid to their own 5′-amino-modified terminus[15]. Here we demonstrate the *in vitro* selection of ribozymes (196 nucleotides) that perform the same peptidyl transferase reaction as the ribosome: that is, they can join amino acids by a peptide bond. Like ribosome substrates, one amino acid (N-blocked methionine) is esterified to the 3′(2′)-O of adenosine, whereas the acceptor amino acid (phenylalanine) has a free amino group. Our best characterized ribozyme recognizes the amino-acid ester substrate by binding its adenosine moiety, and is therefore capable of utilizing Leu- and Phe- as well as Met-derived substrates. Such lack of specificity with respect to the amino acid is a feature necessary for a generalized protein-synthesizing enzyme.

We synthesized a pool of 1.3×10^{15} different RNA molecules containing two regions of randomized sequence (70 and 72 nucleotides) flanked by constant regions. The template for *in vitro* transcription was a synthetic DNA library constructed by using the polymerase chain reaction (PCR) (see Methods). Transcription was performed in the presence of guanosine-5′-monophosphorothioate (GMPS) to introduce a 5′-phosphorothioate. The 196-mer 5′-GMPS-RNA pool was then reacted with *N*-bromoacetyl-*N*′-phenylalanyl-cystamine to convert to 5′-Phe-SS-RNA. Thus, each RNA had a Phe at its 5′ end that provided a free amino group capable of functioning as an aminoacyl acceptor (Fig. 1).

To select active RNA, the 5′-Phe-SS-RNA pool was incubated

with the substrate AMP-Met-Bio (2′(3′)-*N*-biotinylamidocaproyl-L-methionyl-adenosine-5′-monophosphate) in the presence of Mg^{2+} at 25 °C. AMP-Met-Bio is a simple analogue of the natural initiator formyl methionine(fMet)-transfer RNA (Fig. 1). RNA able to form a peptide bond became covalently linked to a biotin tag and was selected by binding to a streptavidin column. The active RNA molecules were eluted from the streptavidin column by reduction of the disulphide linkage with dithiothreitol (DTT). Selected RNAs were reverse-transcribed to complementary DNA, amplified by PCR, transcribed to 5′-GMPS-RNA, and finally converted to 5′-Phe-SS-RNA to enter the next cycle of selection. At the seventh generation, the enriched RNA pool showed a band-shift upon gel electrophoresis that represented a streptavidin–RNA complex (Fig. 2a). The retarded band disappeared after treatment with DTT (Fig. 2a, lane 9c′), which indicated that the biotin was linked to the RNA on the external (Phe side) of the disulphide linker. This suggested that the expected peptide bond had formed between the carbonyl carbon of the amino acid of the substrate (AMP-Met-Bio) and the amino group (phenylalanine) of 5′-Phe-SS-RNA. The active RNA reached 10.4% of the ninth generation RNA pool. After 19 cycles of selection, the activity of the pool RNA had increased markedly, being 1.7×10^4-fold more active than the seventh-generation RNA based on initial rate measurements.

The nineteenth-generation cDNA molecules were cloned. PCR-amplified DNAs from 75 plasmids were used as templates for run-off transcription by T7 RNA polymerase. Kinetic analysis of the individual RNA species revealed that 9 of 75 had better activity than the final RNA pool, while 18 clones were inactive (Fig. 2b). Sequence analysis (24 clones) showed that the most active RNAs comprised at least two sequence classes. The sequence of one of these classes, which included clones 8, 14, 25, 45 and 60, is given as Supplementary information. The deletion of ~20 nucleotides from either the 5′ or the 3′ end, or from both ends of clone-25 RNA decreased its catalytic activity dramatically (data not shown). Therefore, both the 5′ and 3′ constant regions of this ribozyme are necessary for formation of the active structure.

Kinetic analysis with clone-25 RNA and various concentrations of AMP-Met-Bio gave a typical Michaelis–Menten curve (Fig. 3a). The resulting kinetic parameters were: $k_{cat} = 0.05\ min^{-1}$ and $K_m = 190\ \mu M$. Compared with the uncatalysed reaction of *N*-acetylaminoacyl-tRNA transfer with glycine ethyl ester[17], the rate enhancement of the peptide bond formation catalysed by clone-25 RNA is $\sim 1 \times 10^6$. The reaction of clone-25 RNA required Mg^{2+} and reached a maximal rate when $[Mg^{2+}] \geqslant 100$ mM. As with other ribozymes[18], Mg^{2+} may be required for proper RNA folding and may also be involved directly in the chemical step of peptide bond formation. Divalent metal ions also are required for peptidyl transfer to puromycin by the ribosome[19].

To test whether the chemical identity of the linker affected the reaction, it was shortened from 13 to 5 interatomic bonds (Fig. 1 legend). This linker modification of the clone-25 ribozyme abolished activity. Furthermore, the free, linker phenylalanine added *in trans* inhibited the reaction of the clone-25 ribozyme (50% inhibition between 5 and 10 mM), but no inhibition was observed by free phenylalanine or phenylalanine amide. One likely explanation is that the linker provides specific contacts with the RNA that are required for proper positioning of the 5′ amino group of 5′-Phe-SS-RNA in the active site of the ribozyme. Because inhibition by the free linker

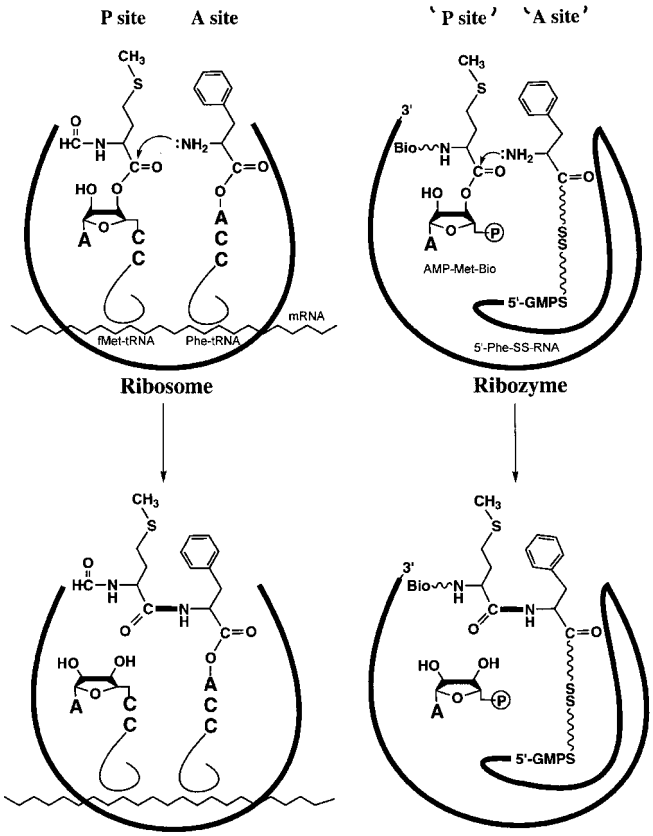

Figure 1 Peptide bond formation by a ribosome (left) and by a ribozyme (right). The process on the ribosome involves two binding sites, the P (peptidyl) and A (aminoacyl) sites. In the ribozyme reaction, 5′-Phe-SS-RNA (functioning like aminoacyl-tRNA in the A site of the ribosome) provides the amino group that can attack the aminoacyl carbonyl carbon of AMP-Met-Bio (mimicking the fMet-RNA or peptidyl-tRNA in the P site of the ribosome) to form a peptide bond (thick line). The standard linker (wavy vertical line) joining GMPS and Phe was $CH_2C(O)NHCH_2CH_2SSCH_2CH_2NH$. One experiment used a shortened linker, SCH_2CH_2NH, attached directly to the 5′ G rather than through the thiophosphate (GMPS).

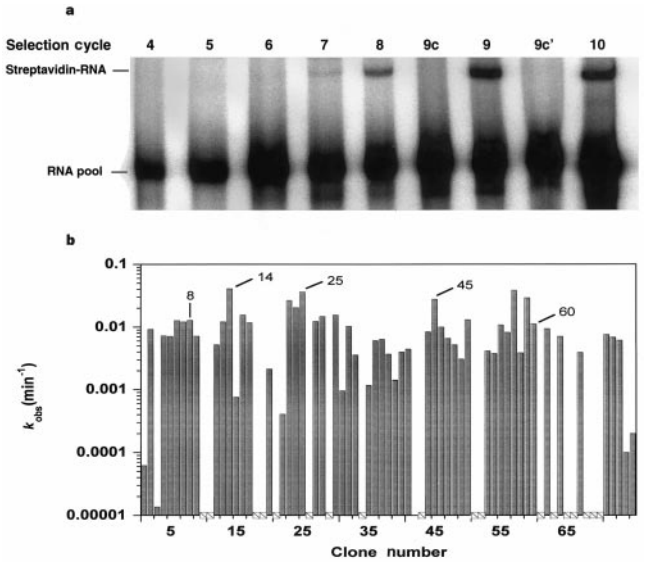

Figure 2 Activity of the selected RNA pool and transcripts from individual clones from generation 19. **a**, Polyacrylamide gel showing the shift of a portion of the radiolabelled RNA after incubation with AMP-Met-Bio and then with streptavidin. Selection reactions were done at 25 °C with 4 μM 5′-Phe-SS-RNA and 8 mM AMP-Met-Bio substrate for 20 h, except for selection cycle 10, which was done with 1 μM 5′-Phe-SS-RNA and 1 mM AMP-Met-Bio for 5 h. Control reactions for pool-9 RNA: lane 9c, no streptavidin; lane 9c′, RNA treated with DTT before streptavidin incubation. **b**, Distribution of the observed rate constants of 75 cloned RNAs. 5′-Phe-SS-RNA (4 μM), prepared from PCR-amplified DNA template by *in vitro* transcription, was incubated with 400 μM AMP-Met-Bio substrate in reaction buffer at 25 °C. RNAs with no detectable activity ($<10^{-6}\ min^{-1}$) are indicated by squares with diagonal line.

requires it to compete with the linker attached intramolecularly to the ribozyme, the interaction energy may be much greater than would be indicated by an apparent K_d of 5–10 mM. In addition, the length of the linker may be critical for allowing the amino group of Phe to reach the active site. Thus, instead of the reactive groups being aligned by local RNA–RNA base pairing, as in many previous ribozyme reactions[8,9,11,13–15,20,21], interactions with the linker may provide alignment.

The peptidyl transferase reaction catalysed by clone-25 RNA was inhibited by adenosine-5′-monophosphate (AMP), but not by other nucleoside-5′-monophosphates. A K_i of about 188 μM for AMP was measured (data not shown). The equivalence of K_m for AMP-Met-Bio and K_i for AMP suggests that most of the substrate binding energy comes from interactions with the AMP, not the amino acid or biotin. The ability of RNA to form specific mononucleotide binding sites is well known[22,23]. Methionine and *N*-formyl-methionine at 1 mM concentration did not inhibit the peptidyl transferase reaction, although higher concentrations of methionine may be required to reveal potential weak interactions between the amino acid of the substrate and the ribozyme.

Several control assays validated the ribozyme-mediated formation of a peptide bond. When 5′-GMPS-RNA (without linked phenylalanine) was incubated with the AMP-Met-Bio substrate under the 5′-Phe-SS-RNA reaction conditions, no product was detected (Fig. 3b, lane 10). Also no product formed without Mg^{2+}, or at zero time of incubation (Fig. 3b, lanes 2 and 7). The disappearance of the product upon treatment with DTT suggested that the biotin group was transferred to the 5′-Phe-SS-RNA externally to the disulphide bond (Fig. 3b, lane 8), consistent with formation of the desired peptide bond. To confirm the formation of the Met-Phe peptide bond, a large-scale reaction was performed to obtain enough material for HPLC and mass spectroscopy. After being released from the RNA by DTT treatment, the RNA-catalysed reaction product had a retention time (23 min) by high-performance liquid chromatography (HPLC) and a mass peak (695 $[M + H^+]$) by mass spectroscopy (MS) identical to those of the chemically synthesized authentic compound (Fig. 4).

Several 2′(3′)-*N*-biotinylamidocaproyl-L-aminoacyl-AMP substrates (Leu, Phe, Met and Lys) were synthesized to evaluate the specificity of the peptide-bond-forming activity of clone-25 RNA. The four substrates were all active, albeit with different reaction rates (Fig. 5). Met and Leu were the best peptidyl donors, and kinetic measurements showed that they reacted with the same rate constant but to different final extents. Lys and Phe were less efficient donors for peptidyl transfer. Although the ribosome catalyses over 20 types of amino-acid transfer reaction during normal protein synthesis, the donor activity of various amino-acid-charged substrates is dependent on the identity of the amino-acid side chain when the tRNA is replaced with smaller RNA fragments[24–26]. In these experiments, the derivatives of acetylated (Ac) Phe were poor donors, and the highest donor activity was observed with derivatives of fMet and AcLeu[24], the same amino-acid preference as for the clone-25 ribozyme reported here.

The peptidyl transferase reaction of the selected ribozymes is fundamentally similar to that carried out by the ribosome (Fig. 1). The 3′(2′)-aminoacyl ester of free adenosine bound by the selected RNA is analogous to the aminoacyl adenosine at the end of tRNA bound in the P site (peptidyl site) of the ribosome. In contrast to the ribosome, the 'A-site' (aminoacyl site) amino acid of the ribozyme is tethered by a non-nucleotide linker. However, we found that the linker provides proper spacing or alignment of the acceptor amino acid (Phe), which appears to be analogous to the role of the A-site tRNA in the ribosome. Although the selected ribozymes contain only ~6% of the number of nucleotides of the contemporary large subunit ribosomal RNA, they also perform a simpler reaction. There is no codon–anticodon recognition on our ribozyme, nor is there translocation. Furthermore, the reaction rates are slow compared to the 15–20 peptide bonds formed per second on a ribosome[17]. Nevertheless, it is now clear that even in a limited portion of RNA sequence space, there are many RNA sequences that possess peptidyl transferase activity.

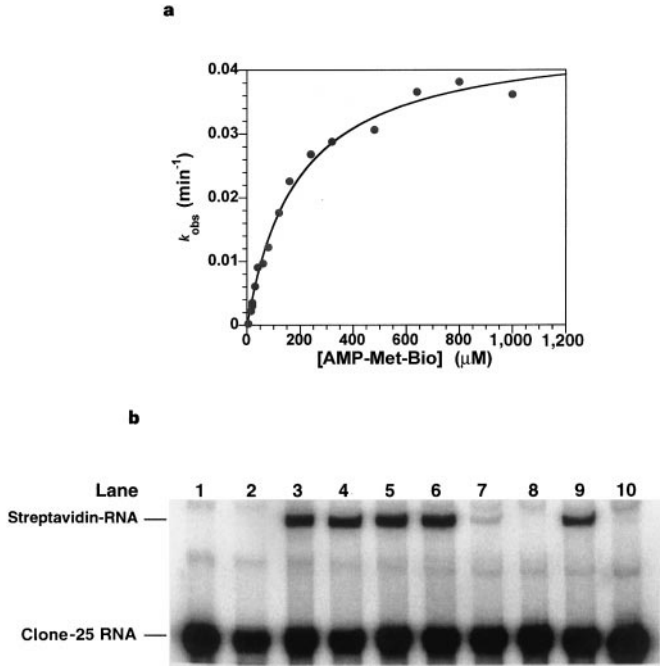

Figure 3 Formation of biotinylated dipeptide catalysed by clone-25 RNA. **a**, Reaction kinetics of clone-25 RNA at 25 °C. Reactions were run in 4 μM 5′-Phe-SS-RNA and 50 mM Mg^{2+} at pH 7.4. Best-fit values of K_m and k_{cat} were obtained by fitting the data to the Michaelis–Menten equation by KaleidoGraph program. **b**, The standard reaction contained 0.4 mM AMP-Met-Bio substrate and 4 μM clone 25 5′-Phe-SS-RNA in 300 mM KCl and 200 mM Mg^{2+}. After 2.5 h at 25 °C, aliquots were removed from the reaction mixture for streptavidin gel-shift assays. Alterations in the standard conditions were: lane 1, no substrate; lane 2, no Mg^{2+}; lane 3, 30 mM Mg^{2+}; lane 4, 60 mM Mg^{2+}; lane 5, 100 mM Mg^{2+}; lane 6, standard reaction; lane 7, zero time; lane 8, 3 μmol DTT; lane 9, 0.1 μmol DTT; and lane 10, 4 μM 5′-GMPS-RNA (no 5′-Phe).

The ribosome may achieve peptidyl transfer simply through precise orientation of substrates, or it may also provide chemical groups to stabilize high-energy oxyanionic intermediates and transition states[17]. Study of the mechanism of peptidyl transfer by *in vitro* selected ribozymes may help reveal the mechanistic features of ribosome-catalysed protein synthesis. □

Methods

Oligonucleotide synthesis. The oligodeoxynucleotides PM1, 5′-AGCGAA TTCTAATACGACTCACTATAGGGAGAGCCATACCTGAC-3′; PM2, 5′-CACGGATCCTGACGACTGAC-3′; N70DNA, 5′-GGGAGAGCCATACCT-GAC-N_{70}-CAGGTTACGCATCC-3′; and N72DNA, 5′-CACGGATCCTGAC-GACTGAC-N_{72}-GGATGCGTAACCTG-3′ were synthesized on an Applied Biosystems DNA synthesizer, model 394. A mixture of phosphoramidites in a ratio 3 : 3 : 2 : 2 (A : C : G : T) was used to synthesize the random positions (N).

Aminoacyl substrate synthesis. AMP-Met-Bio was synthesized by esterification of AMP with *N*-biotinylamidocaproyl-L-methionine[27]. The other AMP-aminoacyl-Bio compounds were similarly synthesized from the corresponding *N*-biotinylamidocaproyl-L-amino acids. The products were purified on a silica gel column eluted with *n*-butanol : acetic acid : water ratio of 78 : 5 : 17 (v/v). The purity of products was confirmed by ^{1}H-NMR and electrospray ionization/MS.

Initial RNA pool. The full-length 222-mer double-stranded (ds) DNA was synthesized by PCR amplification with 120 μg N70DNA and 120 μg N72DNA as templates. PCR with primers PM1 and PM2 was run in a total volume of

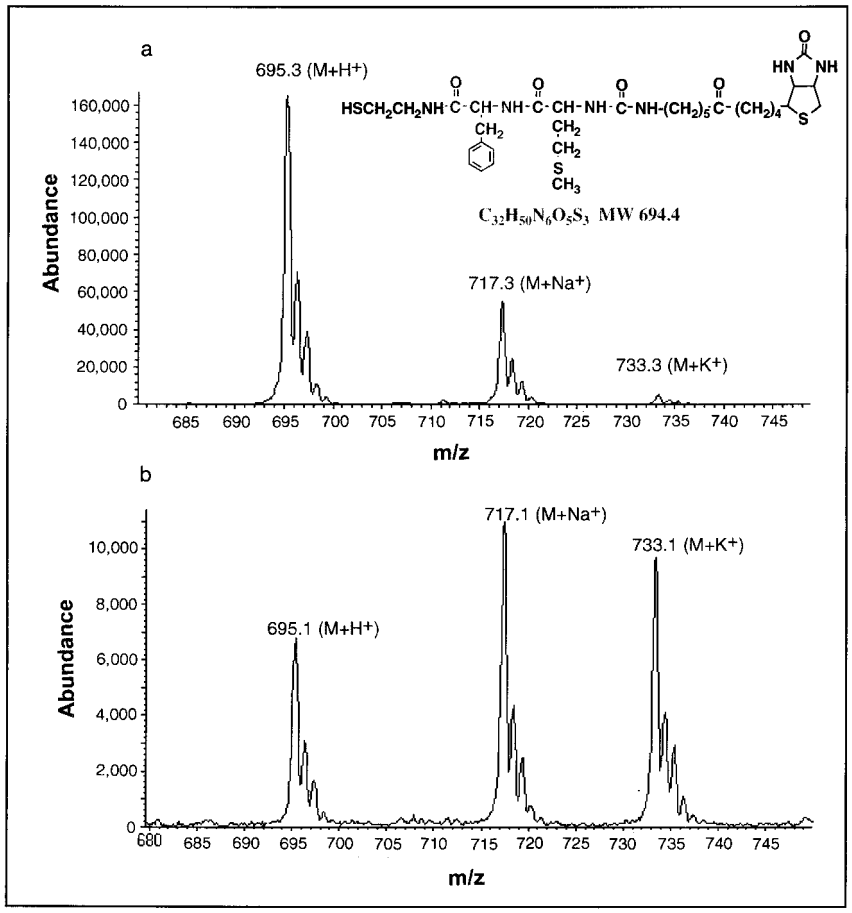

Figure 4 Validation of the peptide bond formed by catalysis of clone-25 RNA by HPLC-ESI/MS analysis of the dipeptide product. **a**, Authentic compound *N*-biotinylamidocaproyl-L-methioninyl-L-phenylalanine-2′-pyridyldithioethyl amide (Bio-Met-Phe-SS-Py) was synthesized by coupling of *N*-biotinylamidocaproyl-methioninyl-*N*-hydroxyl-succinimide ester with *L*-phenylalanine-2′-pyridyl-dithioethyl amide (made by reacting t-Boc-L-phenylalanine-*N*-hydroxyl succinimide ester with pyridyldithioethylamine). This product was then reacted with DTT to give the compound shown. **b**, 5′-*N*-biotinylamidocaproyl-L-methioninyl-L-phenylalaninyl-SS-GMPS-RNA (product of the clone-25 RNA reaction) was also reacted with DTT. The HPLC separation was performed on a Hewlett–Packard 1090 liquid-chromatography system with a C18 reverse-phase column. The elution gradient was 100% water (1 min), 56.7% acetonitrile (35 min) and 100% acetonitrile (41 min). Mass spectrometry was done on a Hewlett–Packard 5989B mass spectrometer. Both the HPLC and MS were controlled by the HP Chemstation software.

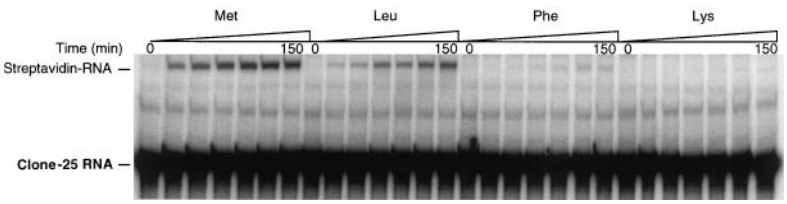

Figure 5 Amino-acid specificity of peptide bond formation catalysed by clone-25 ribozyme. The time course of reactions with 8 μM clone-25 RNA and 500 μM 3′(2′)-*N*-biotinylamidocaproyl-aminoacyl-AMP substrates (Met, Leu, Phe and Lys) in 300 mM KCl, 100 mM $MgCl_2$ and 50 mM HEPES (pH 7.4) at 25 °C.

39.2 ml in 196 tubes, each containing 200 μl of 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 0.01% (w/v) gelatin; 0.05% Tween 20; 0.2 mM dNTPs; 1.0 μM primers and 3.0 mM $MgCl_2$, in 10 cycles (94 °C, 1 min; 52 °C, 1 min; 72 °C, 2 min). The PCR product was extracted once with chloroform : phenol (1 : 1) and once with chloroform : isoamyl alcohol (24 : 1), precipitated with 3.0 volumes of ethanol, resuspended in water, and used for the next step without further purification. The initial RNA pool was prepared from 800 μg PCR-amplified DNA by *in vitro* transcription in a 20-ml reaction mixture (40 mM Tris-HCl, pH 7.5, 10 mM DTT, 4 mM spermidine, 0.05% Triton X-100, 12 mM $MgCl_2$, 1 mM each ATP, CTP, UTP, 8 mM GMPS, 0.4 mM GTP, 100 μCi [α-^{32}P]ATP and T7 RNA polymerase (supplied by A. Gooding)) for 19 h at 37 °C (for the short-linker variant, GMPS was replaced by 5′-deoxy-5′-thioguanosine-5′-monophosphate). 800 U of RNase-free DNase I (Promega) and $CaCl_2$ (final concentration, 10 mM) were added to digest DNA templates. After 2 h, 0.4 ml 0.5 M EDTA (pH 8.0) and 0.6 ml 5.0 M NaCl were added, and the RNA was precipitated with 3.0 volumes of ethanol. The 5′-GMPS-RNA was purified by electrophoresis on a denaturing 8% polyacrylamide gel with 80–90% yield. The 5′-GMPS-RNA (50 μM) was reacted with *N*-bromoacetyl-*N*′-phenylalanyl-cystamine (10 mM) in chemical linking buffer (20 mM HEPES, pH 7.7, 150 mM NaCl, 1 mM EDTA) for 1 h at room temperature and overnight at 4 °C, giving a yield of 40–60%. After phenol extraction, the 5′-Phe-SS-RNA pellet was collected by precipitation with ethanol and used for the selection.

Selection. In the first 9 cycles, 4 μM 5′-Phe-SS-RNA was incubated with 8 mM AMP-Met-Bio substrate in reaction buffer (50 mM HEPES, pH 7.4, 300 mM KCl, 50 mM $MgCl_2$) for 20 h at 25 °C. The reaction mixture was extracted with chloroform : phenol (1 : 1) and chloroform : isoamyl alcohol (24 : 1), and precipitated with 3.0 volumes of ethanol. The pellet was redissolved in 1.0 ml binding buffer (1.0 M NaCl, 20 mM HEPES, pH 7.4, 5 mM EDTA) and transferred to a tube containing 1 ml (5 ml for first cycle selection) of 50% streptavidin–agarose gel slurry (prewashed twice with binding buffer). The slurry mixture was mixed gently for 40–60 min by a roller, loaded on a column, and then washed with 20 ml binding buffer, 20 ml water, 20 ml 7.0 M urea, 20 ml water. Bound RNA was eluted with 3.0 ml 100 mM DTT in binding buffer. The eluted RNA was precipitated with 100 μg glycogen and 2.5 volumes of ethanol. The eluted RNA was converted to cDNA by reverse transcription. A 20-μl annealing mixture (40 mM Tris-HCl, pH 8.3, 8.0 mM DTT, 48 mM NaCl, 5 pmol 3′-primer) was heated for 90 s at 90 °C and slowly cooled to room temperature. Then 50 μl of reverse transcription (RT) mixture (20 μl annealing mix, 0.4 mM dNTPs, 40 mM Tris-HCl, pH 8.3, 8.0 mM DTT, 48 mM NaCl, 2.4 mM $MgCl_2$, 4 U of avian myeloblastosis virus reverse transcriptase) was incubated for 30 min at 40 °C. The cDNA was amplified by PCR in a 100-μl reaction (50 μl RT reaction mix: 2.4 mM $MgCl_2$, 23 mM Tris-HCl, pH 8.3, 12.5 mM KCl, 0.2 mM dNTPs, 1.0 μM primers, 5 U *Taq* DNA polymerase) and purified as described, transcribed, and linked with phenylalanine for the next selection. In subsequent selection cycles, 1–2 μM 5′-Phe-SS-RNA and 200–400 μM AMP-Met-Bio were incubated for 1–5 h; other conditions were the same as those already described.

Kinetic studies. All reactions were carried out with uniformly [α-^{32}P]ATP-labelled ribozyme and excess AMP-aminoacyl-Bio substrate (≥100-fold) in 50 mM HEPES (pH 7.4), 300 mM KCl and 50 mM $MgCl_2$ at 25 °C. The RNA was preincubated with buffer and Mg^{2+} for 10 min at 50 °C, and cooled to room temperature (~20 min). The reaction was initiated by addition of AMP-aminoacyl-Bio substrate. At each time point, 7 or 8 aliquots of 2 μl were removed from 20 μl reaction mixtures and quenched in dry ice with one volume of 'stop' buffer containing 100 mM HEPES (pH 7.4) and 100 mM EDTA. Samples were incubated with 10 μg streptavidin in binding buffer at room temperature before mixing with 0.25 volumes of 90% formamide loading buffer. Biotinylated RNA products were separated by electrophoresis on 8% polyacrylamide/7 M urea gels (1,000 V at 4 °C for 2 h). The fraction of product formation relative to total substrate and product at each time point was quantified with a Molecular Dynamics PhosphorImager. Reactions reached 30–50% completion and rates were corrected for this end point.

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correction

Synthesis of epothilones A and B in solid and solution phase

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We wish to add that, as well as a total synthesis of epothilone B, reference 19 includes biological data for compound **23** and other congeners similar to those reported in our Letter. □

retraction

High levels of genetic change in rodents of Chernobyl

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We previously reported an elevated DNA substitution rate in the mitochondrial cytochrome *b* gene of voles from Chernobyl. Our conclusion was based on a higher level of variation in rodents living in the zone of radioactivity than in rodents from control regions. Those DNA sequences were obtained by manual sequencing of cloned PCR products. We have subsequently attempted to replicate these findings with direct, automated sequencing of PCR products from the same animals. We found discrepancies between these sequences generated by direct automated methods and those previously reported. Re-examination of the original autoradiograms revealed that the elevated levels of variation were largely a product of human error in scoring, aligning and recording the sequences. This error has been confirmed by automated sequencing of a selected sample of the original clones on which the manual sequences were based. There remains a higher rate of substitution in the nine animals from the radioactive zone than in the ten animals from the control population, but the difference is not statistically significant with this sample size. There is variation among the clones from the mice living in the radioactive regions, including clones from the embryos. The two mutations reported in the embryos do not qualify as substitutions based on the standard set forth in the methods and materials that changes be present in multiple clones. The significance (if any) of this variation remains to be determined. The data do not support a mutation rate as high as that reported, and we retract that conclusion. □