10. P. M. Macdonald and G. Struhl, *Nature* **336**, 595 (1988).
11. D. St. Johnston, W. Driever, T. Berleth, S. Richardson, C. Nüsslein-Volhard, *Development (Cambridge)* **107** (suppl.), 13 (1989).
12. P. M. Macdonald, *ibid.* **110**, 161 (1990); M. A. Seeger and T. C. Kaufman, *EMBO J.* **9**, 2977 (1990).
13. M. D. Pondel and M. L. King, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7612 (1988).
14. J. K. Yisraeli, S. Sokol, D. A. Melton, *Development (Cambridge)* **108**, 289 (1990).
15. J. N. Dumont, *J. Morphol.* **136**, 153 (1972).
16. P. A. Krieg and D. A. Melton, *Methods Enzymol.* **155**, 397 (1987).
17. D. A. Melton and R. Cortese, *Cell* **18**, 1165 (1979).
18. M. Jamrich, J. Mahon, E. Gavis, J. Gall, *EMBO J.* **3**, 1939 (1984).
19. P. A. Krieg and D. A. Melton, *Nucleic Acids Res.* **12**, 7057 (1984).
20. The distribution of the injected XβG-Vg3'UTR RNA in the animal and vegetal hemispheres at day 0 (Fig. 1B, lanes 5 and 6) is a random occurrence due to the relatively small pool of oocytes assayed for that time. In addition, the assay results after culture for a single experiment should not be taken to provide a quantitative assessment of the ability of a construct to direct localization but rather are representative of the results obtained for a particular sequence. The extent of localization seen in this experiment was significantly greater than was routinely obtained, although in every case localization was observed.
21. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1989).
22. Reproducible localization was assigned only to those constructs that were consistently localized in the multiple pools of oocytes assayed after culture in each experiment. In side-by-side comparisons, the constructs XβG-366 and XβG-340/3' were localized as much or more than construct XβG-Vg3'UTR.
23. We thank the members of the Melton laboratory for helpful advice and discussions, P. Klein and J. Clifton for comments on the manuscript, and J. Yisraeli both for providing the XβG-Vg3'UTR construct and for instruction on the oocyte culture system. K.L.M. is a fellow of the Helen Hay Whitney Foundation. Supported by grant GM 32921 from the NIH.

17 September 1991; accepted 5 December 1991

The Specificity of Translational Control Switched with Transfer RNA Identity Rules

M. GRAFFE, J. DONDON, J. CAILLET, P. ROMBY, C. EHRESMANN, B. EHRESMANN, M. SPRINGER*

The interaction of *Escherichia coli* threonyl–transfer RNA (tRNA) synthetase with the leader sequence of its own messenger RNA inhibits ribosome binding, resulting in negative translational feedback regulation. The leader sequence resembles the substrate ($tRNA^{Thr}$) of the enzyme, and the nucleotides that mediate the correct recognition of the leader and the tRNA may be the same. A mutation suggested by tRNA identity rules that switches the resemblance of the leader sequence from $tRNA^{Thr}$ to $tRNA^{Met}$ causes the translation of the threonyl-tRNA synthetase messenger RNA to become regulated by methionyl-tRNA synthetase. This identity swap in the leader messenger RNA indicates that tRNA identity rules may be extended to interactions of synthetases with other RNAs.

THE EXPRESSION OF THE GENE FOR *E. coli* threonyl-tRNA synthetase, *thrS*, is negatively autoregulated at the translational level (*1, 2*). The threonyl-tRNA synthetase (ThrRS) binds to the region of the *thrS* mRNA leader that has structural analogies with the natural substrate ($tRNA^{Thr}$) of the enzyme (*3, 4*). The synthetase binds preferentially to tRNA and, if the cellular concentration of the primary substrate decreases, secondarily to the mRNA (*5*). This regulatory scheme is reminiscent of that of the translational feedback mechanism of ribosomal proteins (*6*). We use tRNA identity rules (*7*) to show that ThrRS interacts with stable RNA and mRNA in a similar fashion.

The *thrS* mRNA leader (Fig. 1) is composed of four domains (*4*). Mutational analysis indicated that only domains 2 and 4 are directly involved in the control of *thrS* expression (*8*). These two domains bind directly to the synthetase and compete with $tRNA^{Thr}$ for this binding (*5*). Therefore, we compared the consensus structure of the four $tRNA^{Thr}$ isoacceptors of *E. coli* with domains 2 and 4 of the *thrS* mRNA leader (Fig. 1) (*9*). The nucleotides that mediate the correct recognition of the isoacceptor set by ThrRS define the identity elements. Experiments have shown that the BGU (B stands for C, G, or U) anticodon is involved in $tRNA^{Thr}$ identity (*10, 11*). Theoretical evidence suggests that base pairs U(68)-A(5) and G(71)-C(2) (Fig. 1) also belong to the identity set (*12*). Domains 2 and 4 of the *thrS* mRNA leader (Fig. 1) contain the nucleotides that appear to define $tRNA^{Thr}$ identity at equivalent places. Moreover, an ACCA sequence that forms the 3' end of the tRNA is located at an equivalent position in the leader mRNA. Therefore, it appears that the mRNA leader of *thrS* might have the identity of $tRNA^{Thr}$. We postulated that the identity of the mRNA leader could be changed from $tRNA^{Thr}$ to that of another tRNA, causing control of the *thrS* gene to be dependent on another synthetase.

We decided to change the CGU ($tRNA^{Thr}$ anticodon) to CAU ($tRNA^{Met}$ anticodon) in the mRNA leader equivalent of the anticodon for the following reasons. (i) The principal identity element of both tRNAs appears to be the anticodon. (ii) Clones that overproduce methionyl-tRNA synthetase (MetRS) were available (*13*). (iii) We have isolated the desired mutation as a constitutive mutant that has no effect on the structure of the mRNA leader (*3, 4*).

We introduced a second mutation in the ribosomal binding site (RBS) of the *thrS* mRNA leader. Although this second mutation was not involved in the identity change, we introduced it to increase the sensitivity of *thrS* expression to regulation. The RBS is indirectly involved in control of *thrS* expression because the synthetase competes with the ribosome for binding to the mRNA (*5*). Thus, if the affinity of the ribosome for the mRNA is lowered because of an RBS mutation, the synthetase binding to the mRNA is not inhibited, allowing regulation over a greater range. Conversely, if the affinity of the ribosome for the RBS is increased, a concomitant decrease in regulation is observed (*8*). Therefore, any effect of MetRS

Table 1. The effect of *thrS* mRNA leader mutations on the repression caused by ThrRS (plasmid pUB4) or MetRS (plasmid pNAV7) compared to a pUC12 control plasmid. The β-galactosidase values expressed from the fusions are given in Miller units per absorbance at 650 nm (A_{650}) of bacteria (*19*) and are the averages ± the standard deviation of at least four measurements made between 0.2 and 0.4 A_{650}. The *thrS-lacZ* fusions are indicated as the name of the λ bacteriophages that carry them. The sequence of the mRNA leader counterpart of the anticodon and the corresponding amino acid are given next to the name of the fusion.

thrS-lacZ fusion	Expression of β-galactosidase		
	pUC12	pUB4	pNAV7
λMΔ20-10-XII-25 CGU (Thr)	105 ± 5	17.2 ± 1.3	45 ± 9
λMΔ20-10-XII-25-BS4-9 CAU (Met)	1534 ± 106	1462 ± 97	130 ± 6
λMΔ20-10-XII-25-VII-5 CCU (Arg)	1322 ± 91	1190 ± 79	1475 ± 181
λMΔ20-10-XII-25-VI-1 CUA (Amber)	1757 ± 56	1611 ± 83	1752 ± 20
λMΔ20-10-XII-25-JC4-2 UUG (Gln)	1706 ± 73	1557 ± 39	1890 ± 293
λMΔ20-10-XII-25-ML2 Δ(−21 to −39)	3254 ± 123	3130 ± 153	3107 ± 362

M. Graffe, J. Dondon, J. Caillet, M. Springer, Institut de Biologie Physico-Chimique, 75005 Paris, France.
P. Romby, C. Ehresmann, B. Ehresmann, Institut de Biologie Moléculaire et Cellulaire, 67084 Strasbourg, France.

*To whom correspondence should be addressed.

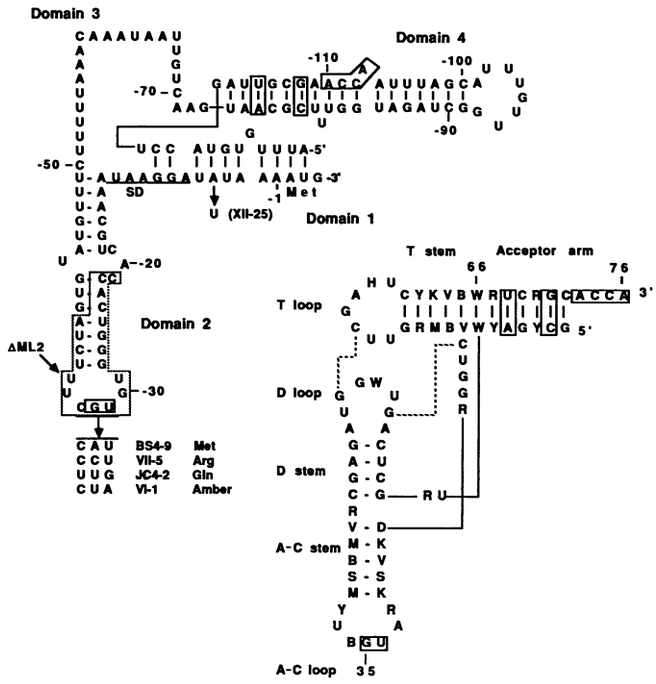

Fig. 1. The *thrS* leader mRNA and the composite structure of the $tRNA^{Thr}$ isoacceptors in the L-shaped representation. The *thrS* leader between +3 and −130 (+1 is the A of the AUG initiation codon) is shown in the upper left part of the figure in a secondary structure compatible with other data (*4*). The Shine-Dalgarno sequence is shown as SD and the NH_2-terminal amino acid, Met, is indicated under the AUG initiation codon. The composite structure of the four known $tRNA^{Thr}$ isoacceptors is shown in the lower right part of the figure, and the seven domains are indicated. The solid lines in both structures connect nucleotides that are linked with 3′,5′-phosphodiester bonds, and the dashed lines connect bases that form base tertiary hydrogen bonds. R stands for A or G; Y for C or U; M for A or C; K for G or U; S for C or G; W for A or U; H for A, C, or U; B for C, G, or U; V for A, C, or G; and D for A, G, or U. The nucleotides thought to be involved in identity are enclosed in solid boxes in both the tRNA and mRNA, as well as the ACCA sequence that forms the 3′ end of the tRNA. Point mutations are indicated with their corresponding names. The mutations in the mRNA leader counterpart of the anticodon are followed by the corresponding amino acids. The deleted nucleotides in the mRNA, ΔML2, are enclosed in a dashed box.

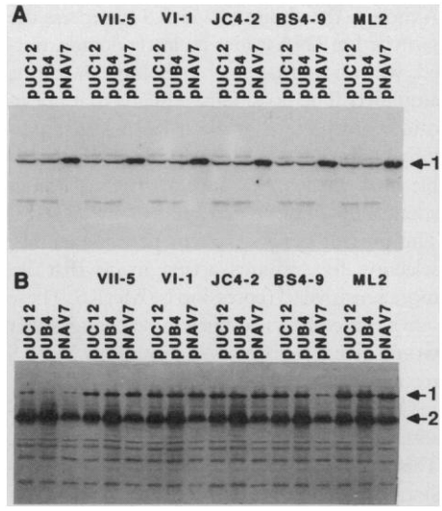

Fig. 2. MetRS and ThrRS amounts in the fusion-carrying strains of Table 1. Autoradiograms of protein immunoblots of samples taken from the same cultures from which β-galactosidase activities were determined (Table 1) with serum raised against *E. coli* MetRS (**A**) or ThrRS (**B**). The extract of samples containing pNAV7 was diluted ten times in (A) only. The MetRS band is indicated at arrow 1 in (A), the ThrRS–β-galactosidase hybrid band is indicated at arrow 1 in (B), and the ThrRS band is at arrow 2 in (B). The name of the respective plasmids and mRNA leader mutants are indicated over each lane. Technical details are as described (*20*).

on the mutated mRNA leader should be increased if the affinity of the ribosome for the RBS is decreased. In a systematic search for RBS mutations that increase control, we identified the XII-25 mutation located between the Shine-Dalgarno sequence and the AUG of *thrS* (Fig. 1) (*8*). The molecular mechanism by which this mutation decreases expression and increases regulation is not known. Thus, we decided to look at the effect of this mutation, along with the CGU (Thr) to CAU (Met) change, on the regulation of *thrS* expression by MetRS.

We transformed strains lysogenized with λ bacteriophages carrying *thrS-lacZ* fusions bearing the different mRNA leaders with pUC12 (a control plasmid), pUB4 (a plasmid expressing ThrRS from the *lac* promoter), and pNAV7 (a plasmid expressing MetRS from its own promoter and the *lac* promoter) and measured β-galactosidase activities in these cultures during exponential growth (*14*). ThrRS represses expression of β-galactosidase from the fusion containing only the XII-25 mutation (Table 1), and MetRS has a weak, but reproducible, negative effect on this fusion. The β-galactosidase expression from the double mutant (XII-25/CGU → CAU) is insensitive to changes in ThrRS concentration but is repressed more than tenfold by an excess of MetRS (Table 1). The β-galactosidase synthesis from other *thrS-lacZ* fusions carrying the XII-25 RBS mutation, with a CGU to CCU change (the $tRNA^{Arg}$ anticodon equivalent), a CGU to CUA change (the amber suppressor tRNA anticodon equivalent), or a CGU to UUG change (the $tRNA^{Gln}$ anticodon equivalent), is insensitive to either a ThrRS or MetRS increase (Table 1). The same is true of a fusion carrying a deletion in the mRNA leader counterpart of the anticodon arm (Table 1) (*15*).

We performed protein immunoblot analysis on all strains to measure the amount of each synthetase in the experiment (Fig. 2). The extract of samples containing pNAV7 was diluted tenfold, accounting for the disappearance of the contaminating bands seen in the other lanes (Fig. 2). We estimate that the synthesis of MetRS from pNAV7 is about 50-fold that of the chromosomal copy of the gene, whereas that of ThrRS from pUB4 is about twofold higher than from the chromosomal *thrS* copy (*16*). The large excess of MetRS produced by pNAV7 probably explains the strong repression found in the double XII-25/CGU → CAU mutant when compared to the control (XII-25 alone repressed by ThrRS) (Table 1).

Thus, the higher repression found with MetRS and the switched mRNA leader reflects the large excess of repressor and not increased mRNA leader sensitivity. The XII-25/CGU → CAU mutant is not optimally recognized by MetRS because there is 15-fold greater synthesis of β-galactosidase from this fusion in the presence of pUC12, as compared to that from the XII-25 control fusion with the same plasmid. This difference probably arises because the XII-25 fusion is efficiently repressed by the amount of ThrRS provided from the chromosomal copy of *thrS*, whereas the XII-25/CGU → CAU fusion is not as efficiently repressed by the endogenous concentration of MetRS.

All the regulatory phenomena observed by measurements of β-galactosidase activity are also observed on the ThrRS–β-galactosidase hybrid protein synthesized from the *thrS-lacZ* fusion gene (Fig. 2B). This indicates that we are measuring effects on gene expression and not on the activity of the hybrid ThrRS–β-galactosidase protein. The same experiments performed in the absence of the XII-25 RBS mutation gave equivalent results, although the repression due to either synthetase was weaker than that measured in the presence of the RBS mutation.

The results presented here indicate that the specificity of regulation of *E. coli thrS* expression can be switched by changing a single nucleotide in the mRNA leader counterpart of the anticodon. A second change in the RBS was added to increase the sensitivity of *thrS* expression to the binding of MetRS.

Although the ability of MetRS to repress the switched mRNA leader is clearly demonstrated, we believe that the repression can still be amplified. The possibility remains that nucleotides not present in the mRNA leader participate in $tRNA^{Met}$ identity. It is also possible that nucleotides in the mRNA leader specifically prevent recognition by MetRS. The present genetic system permits an easy selection for mutants acting in cis that are more sensitive to repression by MetRS. These selections could yield nucleotide changes that would give us information about how MetRS recognizes its tRNA isoacceptors.

Our results indicate that an excess of MetRS somewhat represses wild-type *thrS* expression. This repression is seen in the decrease of expression of *thrS-lacZ* fusions (Table 1) and on protein immunoblots (Fig. 2B), where the band corresponding to the endogenous ThrRS is weaker in the presence of pNAV7 than in the presence of pUC12. Because this effect is lost with changes other than the CGU → CAU change, it appears that the wild-type *thrS* mRNA leader is weakly but specifically recognized by MetRS. Thus, the *thrS* mRNA leader may be recognized with lower affinity by MetRS and possibly other synthetases. The biological relevance of this cross talk remains to be determined.

The present data, coupled with genetic (*10*) and biochemical (*5*) analyses of the *thrS* mRNA leader–ThrRS complex, show that the synthetase recognizes both RNAs by a similar mechanism. Because the three-dimensional structure of the synthetase-tRNA interaction is under analysis, this system should provide a mechanism for studying the three-dimensional structure of an mRNA leader complexed with a translational repressor. The ability to change the regulatory specificity of *thrS* by changing the mRNA leader may make three-dimensional analysis more facile. The specificity of the mRNA leader could be changed to an *E. coli* synthetase for which the three-dimensional interaction with its cognate tRNA is available (*17*). Also, the ability to change the regulation specificity of *thrS* provides the best indication of mimicry between a regulatory site on an mRNA and another site on a stable RNA, to which a single control protein binds to both in order to perform a specific cellular activity.

REFERENCES AND NOTES

1. P. Lestienne, J. A. Plumbridge, M. Grunberg-Manago, S. Blanquet, *J. Biol. Chem.* **259**, 5232 (1984).
2. M. Springer *et al.*, *J. Mol. Biol.* **185**, 93 (1985).
3. M. Springer, M. Graffe, J. S. Butler, M. Grunberg-Manago, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4384 (1986).
4. H. Moine *et al.*, *ibid.* **85**, 7892 (1988).
5. H. Moine *et al.*, *J. Mol. Biol.* **216**, 299 (1990).
6. L. Lindahl and J. M. Zengel, *Annu. Rev. Genet.* **20**, 297 (1986).
7. J. Normanly and J. Abelson, *Annu. Rev. Biochem.* **58**, 1029 (1989).
8. M. Springer *et al.*, unpublished data.
9. Y. Komine, T. Adachi, H. Inokuchi, H. Ozeki, *J. Mol. Biol.* **212**, 579 (1990).
10. M. Springer, M. Graffe, J. Dondon, M. Grunberg-Manago, *EMBO J.* **8**, 2417 (1989).
11. L. H. Schulman and H. Pelka, *Nucleic Acids Res.* **18**, 285 (1990).
12. W. H. McClain and H. B. Nicholas, Jr., *J. Mol. Biol.* **194**, 635 (1987).
13. P. H. Hirel *et al.*, *Biochimie* **70**, 773 (1988).
14. We made the different bacteriophages (with the exception of λMΔ20-10-XII-25–ML2) used in Table 1 by cloning the Hind III–Eco RI insert of M13mp8Δ20-10-XII-25 (or its derivatives) between the left arm of λSKS107 (to its Hind III site) and the right arm of λgt4 from its Eco RI site, as described (*2*). This cloning reconstitutes the *thrS-lacZ* hybrid in its integrity. The phage λSKS107 is *imm21* and *nin5*, and we made it by cloning the Hind III–Sst I fragment of pSKS107 [S. K. Shapiro, J. Chou, F. V. Richaud, M. J. Casadaban, *Gene* **25**, 71 (1983)] that carries the 5′ terminal part of *lacZ* without a translation initiation site between the left arm of λnav8-5 (to its Sst I site) and the right arm of λNM540 (from its Hind III site) as described (*2*). M13mp8Δ20-10-XII-25 is an M13mp8 derivative carrying an insert extending from the Pst I site 1194 bp in front of *thrS* to a reconstituted Hind III site 53 bp on the 3′ side of the ATG of *thrS*. The insert also carries the XII-25 mutation (Fig. 1) that was introduced with oligonucleotide site-directed mutagenesis, as was done for all the other mutations of the *thrS* mRNA leader. The phage λMΔ20-10-XII-25–ML2 was selected from a λMΔ20-10-XII-25 lysogen of *E. coli* IBPC5421 as conferring a Lac^+ phenotype (*3, 8*). Strain IBPC5421 was lysogenized with a single copy of the recombinant phages. The F′*lacI*^{q}Tn*10* episome was introduced in the single lysogens by conjugation. The male lysogens were then transformed with pUC12, pUB4 (*2*), or pNAV7 (*13*). The strains were grown in MOPS-glucose medium, supplemented with all amino acids (*18*), tetracycline at 10 μg/ml, and isopropyl-1-thio-β-D-galactoside (IPTG) at 10^{-3} M. We added ampicillin at a concentration of 100 μg/ml at 1-hour intervals during growth to select for the presence of the plasmids.
15. High expression from the fusions mutated in the mRNA leader equivalent of the anticodon is due to the inability of the endogenous ThrRS to repress β-galactosidase synthesis from the *thrS-lacZ* fusions.
16. Low production of ThrRS by pUB4 is probably due to the repression of *thrS* expression from the *lac* promoter in the presence of glucose or the presence of the F′*lacI*^{q}Tn*10* episome. The episome somewhat represses *thrS* expression even in the presence of IPTG. The F′*lacI*^{q}Tn*10* is necessary because pNAV7 is lethal to the cells when MetRS is expressed from both the *lac* and its own promoter but not when expressed only from the latter.
17. M. A. Rould, J. J. Perona, D. Söll, T. A. Steitz, *Science* **246**, 1135 (1989).
18. F. C. Neidhardt, P. L. Bloch, S. Pedersen, S. Reeh, *J. Bacteriol.* **129**, 378 (1977).
19. J. H. Miller, *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1972).
20. J. S. Butler, M. Springer, J. Dondon, M. Grunberg-Manago, *J. Bacteriol.* **165**, 198 (1986).

21. This work is dedicated to the memory of G. Fayat. We thank G. Fayat and C. L. Olsson for discussions; Y. Mechulam for the pNAV7; C. L. Olsson, E. N. Brody, M. Nomura, and P. Shimmel for reading the manuscript; and M. Grunberg-Manago and J. P. Ebel for their support and interest. Supported by grants from the CNRS (UA 1139 and LP6201), INSERM (891017 to M.S.), the European Economic Community [SC1*/0194-C(AM) to M. Grunberg-Manago], and the Fondation pour la Recherche Médicale (to M. Grunberg-Manago).

8 July 1991; accepted 10 October 1991

Reversal of Diabetes Insipidus in Brattleboro Rats: Intrahypothalamic Injection of Vasopressin mRNA

GUSTAV F. JIRIKOWSKI,* PIETRO PAOLO SANNA, DOMINIQUE MACIEJEWSKI-LENOIR, FLOYD E. BLOOM

Messenger RNAs occur within the axons of magnocellular hypothalamic neurons known to secrete oxytocin and vasopressin. In Brattleboro rats, which have a genetic mutation that renders them incapable of vasopressin expression and secretion and thus causes diabetes insipidus, injection into the hypothalamus of purified mRNAs from normal rat hypothalami or of synthetic copies of the vasopressin mRNA leads to selective uptake, retrograde transport, and expression of vasopressin exclusively in the magnocellular neurons. Temporary reversal of their diabetes insipidus (for up to 5 days) can be observed within hours of the injection. Intra-axonal mRNAs may represent an additional category of chemical signals for neurons.

THE MRNAS ENCODING THE HORmones arginine vasopressin (AVP) and oxytocin (OT) are present in axons of the hypothalamoneurohypophysial tract (*1–3*). Oxytocin mRNA has been localized by in situ hybridization and electron microscopy in large granular vesicles of axonal varicosities in the lateral hypothalamus, the median eminence, and the posterior pituitary (*2*). Furthermore, the amounts of OT mRNA in the hypothalamoneurohypophysial tract change with functional demand on the neurons (*4*).

The presence of a neuropeptide mRNA in vesicles and evidence for its axonal transport (*2*) suggest that this mRNA could provide an intraneuronal or intercellular signal. Because of the low activity of brain ribonuclease (RNase) (*5*), we were able to investigate the metabolic fate of exogenous AVP mRNA injected into the axonal fields of these magnocellular neurons. To do so, we used the Brattleboro rat, which has a single base deletion in exon B of the propresso-

Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

*To whom correspondence should be addressed.