UGA NONSENSE SUPPRESSION ASSAYED BY T4 DNA-DEPENDENT IN VITRO SYNTHESIS OF LYSOZYME

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Summary: Suppression of a UGA nonsense mutation was shown using the Gold and Schweiger (14,15) T4 DNA-dependent system for the synthesis of lysozyme in vitro. In extracts of non-permissive cells, suppression was completely dependent on addition of added Su^{+}_{UGA} tRNA from a known UGA suppressor strain; Su^{-} tRNA did not support the suppression. Control backgrounds without T4 DNA or Su^{+}_{UGA} tRNA were low and stable, perhaps because any reinitiation or non-specific misreading at nonsense codons produced no active lysozyme. Thus, the system is sensitive enough to detect very low levels of suppression.

If the nonsense codons specified by UAG, UAA or UGA appear within the cistron for a polypeptide chain, premature termination of that polypeptide chain occurs at that site (1-5). Suppression of a nonsense mutation can occur when the nonsense codon is recognized, for example by an Su^{+} tRNA, which inserts an amino acid and thereby prevents chain termination (6). Suppression in vitro has been shown with amber UAG Su^{+} tRNA (7, 8, 9, 10) and UGA Su^{+} tRNA (11).

Most in vitro studies on suppression have been done with the R17 RNA system (7, 8, 10, 11). However the lack of an appropriate UAA or UGA mutant in R17 RNA has in great part limited studies to suppression of amber UAG codon. Recently UGA suppression has been demonstrated with Su^{+}_{UGA} tRNA by Model et al (11), but the background was rather high and the procedure quite complex. Gesteland et al (9) showed that in vitro suppression can be demonstrated by addition of extracted mRNA from amber lysozyme mutants of T4 into cell-free extracts. This and the efficient system of T4 DNA-dependent in vitro synthesis developed by Gold and Schweiger (14, 15) seemed like potentially very sensitive ones with which to assay suppression of various nonsense mutations in vitro. In this communication, the Gold and Schweiger system of in vitro T4 DNA-dependent synthesis of lysozyme was used to detect synthesis directed by DNA from a UGA lysozyme mutant.

MATERIALS AND METHODS

T4 UGA lysozyme mutant eL4P41 was a gift of Dr. George Streisinger. E. coli strain CAJ64 UGA Su^+, from the collection of Dr. S. Brenner, was used as the suppressing host and as the source of Su^+_{UGA} tRNA. E. coli strain N463 was used as a non-suppressing Su^- host. It is a derivative of D10 (RNase I^-, met^-), with additional tyr^- and ilv^- markers introduced by nitrosoguanidine mutagenesis; it was the source of ribosomes and soluble enzymes (S100; prepared according to reference 14).

T4 bacteriophage DNA was prepared by the methods of Thomas and Abelson (12). Su^+_{UGA} tRNA was prepared according to Gupta and Khorana (13) with the modification thatprecipitation with isopropyl alcohol was applied after instead of before DEAE-cellulose column chromatography. Ribosomes, S100 and conditions for synthesis of phage specific lysozyme directed by T4 DNA in vitro were essentially the same as those of Gold and Schweiger (14, 15), as was the assay for lysozyme activity (15). Only the elution of S100 differed by elution from DEAE-cellulose with 0.3M instead of 0.25M NH_4Cl as reported by Gold and Schweiger.

All filters used in the lysozyme assays reported had approximately 35,000 cpm labelled cells per filter. After 4 hours of incubation at 37°C, 0.1ml of incubation mixture (with 1 ug of wild type T4 DNA, incubated for 40 minutes at 37°C) released approximately 15,000 cpm from a filter. Control backgrounds without any T4 DNA or under non-permissive conditions showed the non-specific solubilization of 500-700 cts/min in 4 hours.

RESULTS

When eL4P41 T4 DNA containing an UGA nonsense mutation in the lysozyme gene was used to direct in vitro protein synthesis, no lysozyme activity was detected with or without added Su^- tRNA (Figure 1). On addition of Su^+_{UGA} tRNA, lysozyme activity was detectable as measured by the increased solubilization of ^3H-DAPA from cells on filters. Control incubation mixtures without any

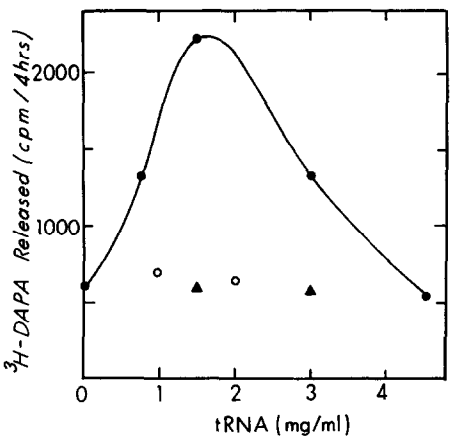

Figure 1. The In vitro suppression of UGA mutant eL4P41 T4 DNA as a function of Su^+_{UGA} tRNA. Protocol of incubation mixtures for T4 DNA directed protein synthesis was essentially the same as those of Gold and Schweiger. 0.1 ml cell free incubation mixtures were incubated for 40 minutes at 37°C, then removed and assayed for lysozyme activity. (See Methods). (●) 0.1 ml incubation mixtures containing 32 µg eL4P41 T4 DNA and varying amounts of Su^+_{UGA} tRNA. (○) 0.1 ml incubation mixtures containing 32 µg eL4P41 T4 DNA and varying amounts of Su^- tRNA. (▲) 0.1 ml incubation mixtures containing no DNA and varying amounts of Su^+_{UGA} tRNA.

T4 DNA, or without Su^+_{UGA} tRNA, showed no lysozyme activity.

Figure 2 shows that suppression was dependent on DNA. Incubation mixtures containing 2250 µg/ml of Su^+_{UGA} tRNA showed an optimum at 63 µg/ml of T4 DNA (Figure 2). Absolute requirements for ribosomes and S100 were also observed. In all cases, control experiments using 2000 µg/ml of Su^- tRNA instead of Su^+ tRNA showed no lysozyme activity at any level of added DNA.

A magnesium optimum of 14 mM was obtained for the activity of Su^+_{UGA} tRNA, while with Su^- tRNA, lysozyme synthesis remained relatively low at all magnesium concentrations from 3 to 20 mM (Figure 3). Again, the results indicate that the lysozyme activity assayed was due to the suppressing capacity of the Su^+_{UGA} tRNA.

DISCUSSION

Here, as in many other suppressor strains of E. coli, Su^+ tRNA is the cellular component responsible for nonsense suppression (7,8,9,10,11). However, this new system can be used relatively more effectively. Its usefulness, in

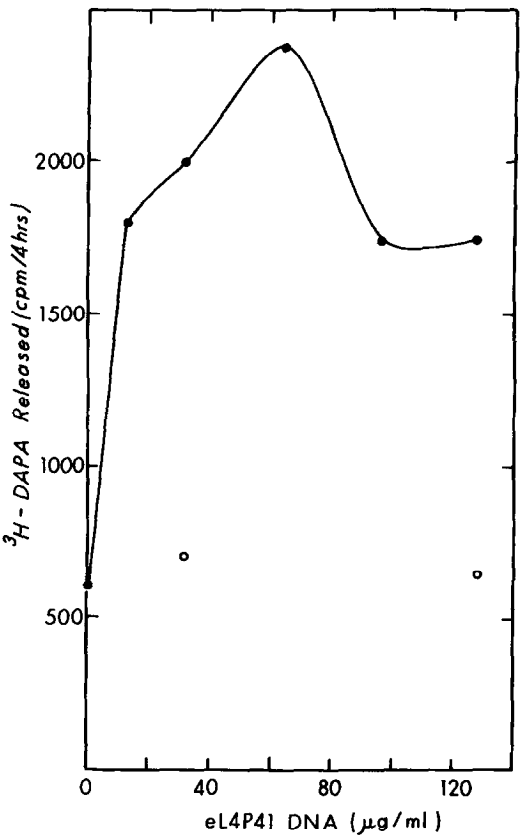

Figure 2. The in vitro suppression of UGA mutant eL4P41 T4 DNA as a function of eL4P41 T4 DNA. Protocol of incubation mixtures for T4 DNA directed protein synthesis as in Figure 1, with 0.1 ml incubation mixtures assayed for lysozyme activity (see Methods). (●) 0.1 ml incubation mixtures containing 225 μg of Su^+_{UGA} tRNA and varying amounts of eL4P41 T4 DNA. (○) 0.1 ml incubation mixtures containing 200 μg of Su^- tRNA and varying amounts of eL4P41 T4 DNA.

contrast to the other systems, is that there is no apparent "leakiness" in Su^- extracts with the lysozyme system. Model et al (11) showed in vitro that op9, a mutant of RNA phage f2 with an UGA mutation in the polymerase gene, directs the formation of about 20-30% as much polymerase protein, detected by its electrophoretic mobility, as does f2 RNA even in the absence of Su^+ tRNA. The high "leakiness" could be explained as non-specific tRNA reading of UGA, resulting in a complete polymerase product; but alternate sources of suppression could not be excluded.

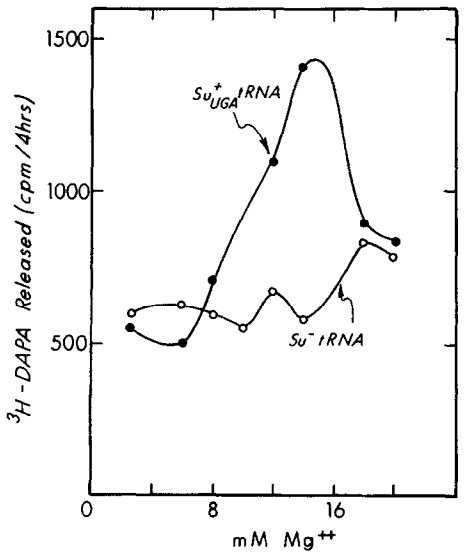

Figure 3. The In vitro suppression stimulated by UGA mutant eL4P41 T4 DNA as a function of magnesium concentration. Protocol of incubation mixtures for T4 DNA directed protein synthesis as in Figure 1. 0.1 ml incubation mixtures were assayed for lysozyme activity (see Methods). Each 0.1 ml incubation mixture contains 6.4 µg eL4P41 T4 DNA, either 150 µg Su^+_{UGA} tRNA (●) or 100 µg Su^- tRNA (○) with varying concentrations of magnesium.

Several possibilities can explain the apparent greater fidelity observed with the lysozyme assay system. Perhaps synthesis with a messenger RNA for an enzyme can be more resistant to "leakiness" than is synthesis with R17 RNA. Alternatively, with the lysozyme system, even if non-specific reading occurs, many non-specific tRNA readings of UGA may give complete but inactive lysozyme molecules. The true, more specific suppressor tRNA reading can insert an amino acid that will give an active lysozyme molecule.

Similar considerations may apply to the observed requirement for magnesium. Capecchi has shown (16) that for the Sus 3 amber mutant of R17 RNA, increasing magnesium concentrations provoke a hugh increase in "leaky" amino acid incorporation under non-permissive conditions. In contrast, for lysozyme synthesis In vitro, the magnesium optimum for suppression is 14 mM (Figure 4), the same as that for lysozyme formation with wild-type DNA

(14, 15); and with increasing magnesium concentrations, there is no great increase in lysozyme activity when Su^- tRNA is supplied to a non-permissive extract.

This is the first case in which the entire process of nonsense suppression starting from DNA, has been possible in cell-free extracts. It may permit further analysis of certain cases in which events at a nonsense codon influence both translation and messenger RNA levels (17).

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REFERENCES

1. Last, J. A., Stanley, W. M., Salas, M., Hille, M. B., Wahba, A.J., and Ochoa, S., Proc. Natl. Acad. Sci. U S. 57, 1062 (1967).
2. Capecchi, M. R., Proc. Natl. Acad. Sci. U.S. 58, 1144 (1967).
3. Bretscher, M.S., J. Mol. Biol. 34, 131 (1968).
4. Brenner, S., Barnett, L., Katz, E. R., and Crick, F.H.C., Nature 213, 449 (1967)
5. Weigert,M. G., Lanka, E., and Garen, A., J. Mol. Biol. 23, 391 (1967).
6. Gorini, L., and Beckwith, J.R., Ann. Rev. Microbiol. 20, 401 (1966).
7. Capecchi, M.R., and Gussin, G.N., Science 149, 417 (1965).
8. Engelhardt, D.L., Webster, R.E., Wilhelm, R. C., and Zinder, N.D., Proc. Natl. Natl. Acad. Sci. U.S. 54, 1791 (1965).
9. Gesteland, R.F., Salser, W., and Bolle, A., Proc. Natl. Acad. Sci. U.S. 58, 2036 (1967).
10. Söll, D., J. Mol. Biol. 34, 175 (1968).
11. Model, P., Webster, R. E., and Zinder, N. D., J. Mol. Biol. 43, 177 (1969).
12. Thomas, C. A., and Abelson, J., in Procedures in Nucleic Acid Research, ed. G. L. Cantoni and D. R. Davies (New York; Harper and Row, 1966), p.553.
13. Gupta, M. K., and Khorana, H. G., Proc. Natl. Acad. Sci. U.S. 56, 772 (1966).
14. Gold, L., and Schweiger, M., Proc. Natl. Acad. Sci. U.S. 62, 892 (1969).
15. Schweiger, M., and Gold, L., Proc. Natl. Acad. Sci. U.S. 63, 1351 (1969).
16. Capecchi, M. R. J. Mol. Biol. 30, 213 (1967).
17. Morse, D. E., and Primakoff, P., Nature 226, 28 (1970).