EFFECT OF 5 FLUOROURACIL ON THE SOLUBLE RNA OF TRICHODERMA

Jonathan Gressel & Esra Galun
Plant Genetics Section, Weizmann Institute of Science
Rehovoth, Israel

Received May 31, 1966

We have recently demonstrated that 5 fluorouracil (FU) suppressed photoinduced sporulation of Trichoderma viride (Pers. ex Fries) cultured on filter paper. This occurred without a detectable inhibition of the growth of the fungus (Galun & Gressel 1966). We have also found that FU, for the duration and concentration used, had but a small effect on labelled amino acid and purine base incorporation into the acid insoluble fractions of the fungus (Gressel & Galun, in preparation). There is ample evidence in the literature that FU can be incorporated into all the fractions of RNA, although the mode of action of FU is interpreted differently for different species (see review by Heidelberger, 1965). It has been reported that FU incorporation into mRNA can 'damage' the genetic code (Champe & Benzer, 1962). There have been various reports of FU induced alterations of RNA sedimentation patterns with modified and displaced rRNA fractions that were less stable than normal (see review by Osawa, 1965, and also Iwabuchi et al, 1965 as well as Andoh & Chargaff, 1965). Our RNA fractionation studies indicate that besides the ribosomal shifts discussed above, there is another new pronounced effect of FU in Trichoderma: an additional peak between the 4S and '5'S sRNA peaks.

METHODS

Spores of T. viride were suspended in water and decanted into Difco potato dextrose broth (48g/l). Flasks were incubated on a shaker at 25° C. After 24 h, cultures were bulked and divided into equal groups with labelled nucleic acid precursors. After incubation, mycelia were filtered and washed with chilled suspending buffer (0.01 M tris pH 7.6, 0.01 M $MgCl_2$, 0.06 M KCl). The mycelia from different treatments were mixed in the cylinder of a chilled French press, suspended in 6 ml suspending buffer and 1 ml 5.5% sodium lauryl sulfate (Duponal) and extruded. The suspension of ruptured cells was repeatedly extracted with phenol until no protein was present in the phenol-buffer interface following centrifugation. RNA was precipitated with ethanol, and dissolved in 0.2N NaCl in 0.05N $NaHPO_4$ buffer (pH 6.7). Aliquots of the dissolved RNA were diluted and applied to methylated albumin Kieselguhr columns (Mandell & Hershey, 1960), and the columns washed with additional 0.2N NaCl + buffer. A linear gradient of 0.2 to 1.2N NaCl in buffer was applied and 4.4 ml fractions were collected. Percent transmission was recorded at 260 mμ during elution. After elution, 200 μg of carrier RNA were added to each tube and trichloroacetic acid (TCA) was added to make a 5% solution, and the tubes were chilled. The RNA was collected on membrane filters, and washed with cold 5% TCA. The filters were dried and covered with toluene:PPO:POPOP (1000:4:0.05, v:w:w) scintillation fluid and radioactivity measured.

RESULTS

Day old cultures of Trichoderma were bulked and divided. Adenosine·H^3 was added to one flask together with unlabelled FU at a concentration that prevented sporulation in petri dishes without overly affecting growth (Galun & Gressel, 1966).

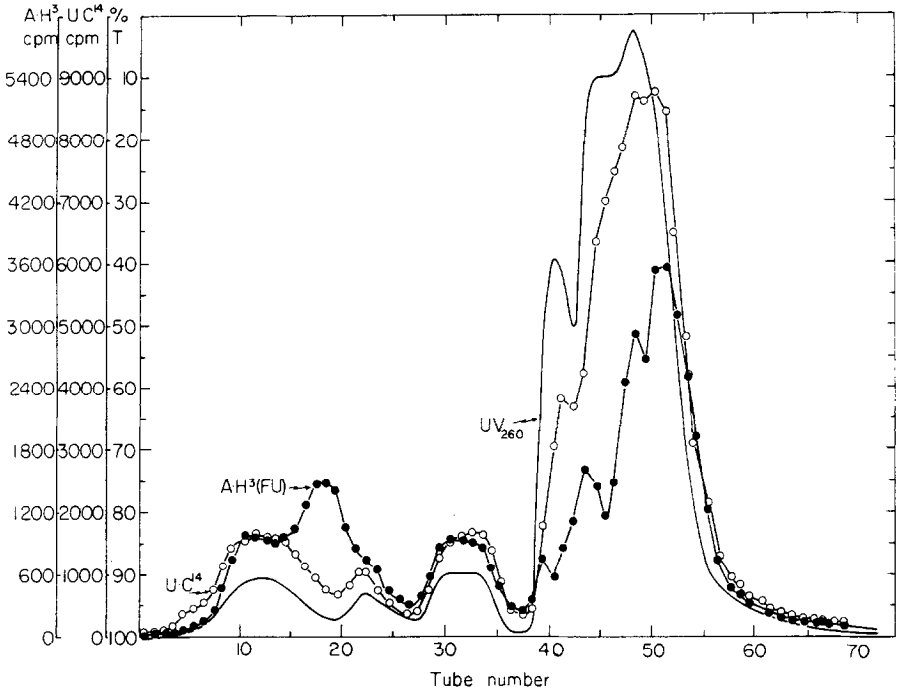
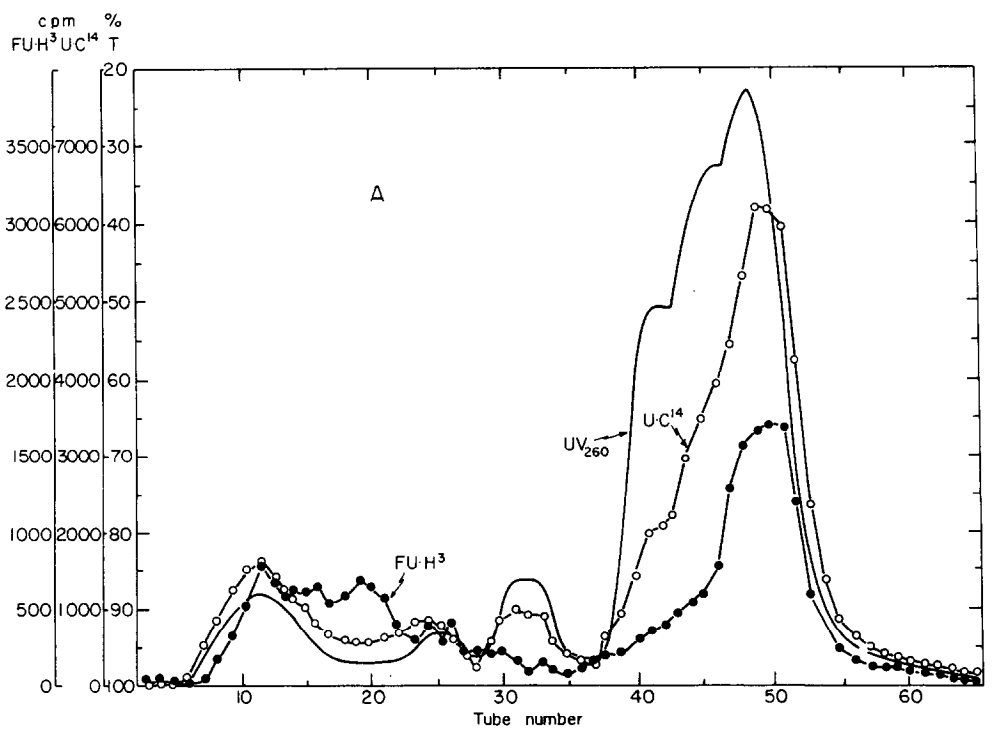


Fig. 1. Influence of FU on the incorporation of adenosine·H^3 into RNA.

Two 200 ml, day old cultures of Trichoderma were bulked and equally divided into two flasks. 200 µC of adenosine·H^3 (uniformly labelled, 2.9C/mmole were added to one flask together with unlabelled FU to give a $2x10^{-4}$M FU solution. Five µC of uracil-2-C^{14} (40mC/mmole) were added to the control flask. Cultures were incubated for 3 h and the RNA prepared as in METHODS.

Uracil·C^{14} was added to the second flask as a control. After a 3 h incubation, the mycelia from both flasks were filtered and washed separately, then mixed and the RNA extracted and chromatographed. The results are plotted in figure 1. The UV curve shows two peaks in the soluble region, the larger of which sedimented in the analytical ultracentrifuge at $4.4S_{20}$. The smaller peak is probably identical with the '5'S peak reported in mammalian cells (Galibert et al., 1965). The DNA peak sedimented at $7.7S_{20}$ in the ultracentrifuge. Two of the three peaks in the ribosomal region sedimented at 17 and $23S_{20}$ and

a third did not appear. Uracil was incorporated in all the peaks of the unlabelled RNA, although, over a long period relatively more uracil was incorporated into the soluble RNA and DNA peaks than into the later eluting RNA. In contrast, FU induced several changes in the incorporation pattern of adenosine·H^3 into RNA. Several small shifts occurred in the ribosomal fractions. Relatively more soluble RNAs than ribosomal RNAs were produced under the influence of FU, and the 4.4S RNA began eluting slightly later than normal. The main difference was the large peak that appeared between the 4.4S RNA and the '5'S RNA. FU did not decrease the relative incorporation of adenosine.H^3 into the DNA peak. To determine whether FU was itself incorporated into the FU·sRNA and to determine whether any of the FU·RNA peaks had a greater relative instability, FU·H^3 was supplied to two of four flasks and uracil·C^{14} to the other two, 24 h after spore innoculation. After a 2 h incubation, unlabelled



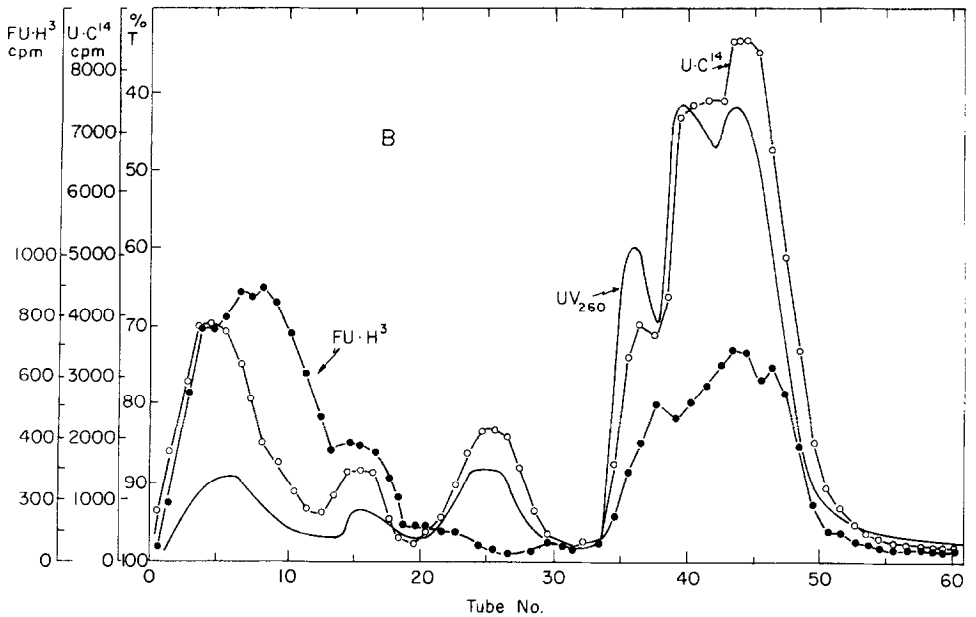


Fig. 2. The incorporation of FU into RNA and the relative stability of the FU·RNAs. A-2 h pulse; B-2 h pulse + 2 h chase.

Two 200 ml, 24 h old cultures of Trichoderma were bulked and equally divided into four flasks. Flasks 1 and 2 received 5μC uracil-2-C^{14} (40mC/mole) and 100μC FU-6-H^3 (580mC/mmole) respectively and were incubated 2 h at which time the RNA was extracted as in METHODS. The results are plotted in 2A. Flasks 3 and 4 were given twice as much uracil-2-C^{14} or FU-6-H^3 as flasks 1 and 2. After a 2 h incubation, unlabelled uracil was added to each flask at 500 times the molar concentrations of uracil·^{14} and FU·H^3. The flasks were then incubated for an additional 2 h and the RNA extracted and chromatographed. The results are shown in 2B.

---

uracil was added to one flask with FU·H^3 and to one with U·C^{14}, at a molar concentration 500 times greater than was added with the isotopes. Incubation was continued for an additional 2 h. The incorporation was stopped in the other two flasks after 2 h and the RNA extracted. The results from the uracil and FU pulses are plotted in Fig. 2A, and the pulses and chase in Fig. 2B. The incorporation of FU·H^3 into RNA (Fig. 2A) was similar to the incorporation of adenosine·H^3 in the presence of unlabelled FU (Fig. 1) except that the FU·H^3 was not incorporated into DNA. Upon hydrolysis of FU·RNA, we have found that all of the label

remains in the FUMP (Gressel & Galun, in preparation). The FU·RNA peaks all have about the same relative stability (Fig. 2B).

The data presented above indicate that in Trichoderma a new type of FU·RNA is formed in great quantity in the soluble RNA region. Some anomalies also exist in the ribosomal region. It also appears that under the influence of FU, relatively more FU·sRNA is manufactured than FU ribosomal RNAs. This difference is not as pronounced as Hignett (1964) has reported for S. aureus. Both the new FU·RNA in the sRNA region and the FU ribosomal RNA appear to have similar relative stabilities.

It is possible to add another feasible mode of action of FU in inhibiting photo-induced sporogenesis to those previously reported, i.e. the production of spurious proteins because of spuriously coded tRNA. This is in addition to the feasibility of inactive proteins being manufactured because of spurious mRNA synthesis. Both of these possibilities take into consideration the fact that there is little reduction in amino acid incorporation during the short period when FU is present. Although normal ribosome production is affected, this cannot explain the FU suppression of sporulation, as sufficient 'old' ribosomes are available for protein synthesis, whereas small amounts of spurious tRNA can falsify the translation process.

ACKNOWLEDGEMENTS

The capable technical assistance of Dina Heller and Cezar Tabbak is gratefully acknowledged. 5 Fluorouracil was a gift of F. Hoffman La Roche and Co., Basle.

REFERENCES

Andoh, T. and Chargaff, E. (1965). Proc. Natl. Acad. Sci. U.S. 54: 1181.

Champe, S.P. and Benzer, S. (1962). Proc. Natl. Acad. Sci. U.S. 48: 532.
Galibert, F., Larsen, C.J., Lelong, J.C. and Boiron, M. (1965). Science 207: 1039.
Galun, E. and Gressel, J. (1966). Science 151: 696.
Heidelberger, C. (1965). In: Progress in Nucleic Acid Research and Molecular Biology, J.N. Davidson and W.E. Cohn, eds. Academic Press, N.Y., Vol. 4, p. 2.
Hignett, R.C. (1964). Biochim. Biophys. Acta 91: 584.
Iwabuchi, M., Otaka, E., Kono, M. and Osawa, S. (1965). Biochim. Biophys. Acta 95: 38.
Mandell, J.E. and Hershey, A.D. (1960). Anal. Biochem. 66: 77.
Osawa, S. (1965). In: Progress in Nucleic Acid Research and Molecular Biology, J.N. Davidson and W.E. Cohn, eds. Academic Press, N.Y., Vol. 4, p. 101.