storage tissue and it contains little alcohol-soluble protein (*16*). An alternative explanation—that there is some passage of ribonuclease from the endosperm into the embryo—gains some support from the observation that the ratio of the activities of *opaque-2* to normal in the embryo reaches a peak several days after that of the endosperm.

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References and Notes

1. Cited by R. A. Emerson, G. W. Beadle, A. C. Fraser, *Cornell Univ. Agr. Exp. Sta. Mem. No. 180* (1935).
2. E. T. Mertz, L. S. Bates, O. E. Nelson, *Science* **145**, 279 (1964).
3. L. S. Bates, in *Proceedings of the High Lysine Corn Conference*, E. T. Mertz and O. E. Nelson, Eds. (Corn Industries Research Foundation, Washington, D.C., 1966), p. 61.
4. J. R. Jimenez, *ibid.*, p. 74.
5. A. Dalby, *ibid.*, p. 80.
6. J. Mossé, *Fed. Proc.* **25**, 1663 (1966); ———, J. Baudet, J. Landry, T. Moureaux, *Compt. Rend.* **263D**, 788 (1966).
7. A. Dalby and J. J. Murphy, in preparation.
8. O. E. Nelson, E. T. Mertz, L. S. Bates, *Science* **150**, 1469 (1965).
9. J. Ingle and R. H. Hageman, *Plant Physiol.* **40**, 48 (1965).
10. T. W. Tuve and C. B. Anfinsen, *J. Biol. Chem.* **235**, 3437 (1960).
11. C. M. Wilson, *Biochim. Biophys. Acta* **68**, 177 (1963). Our unit is one-fifth of the Wilson unit because of a fivefold difference in final reaction volume.
12. J. Ingle, *ibid.* **73**, 331 (1963).
13. ———, D. Beitz, R. H. Hageman, *Plant Physiol.* **40**, 835 (1965).
14. R. K. Morton and J. K. Raison, *Biochem. J.* **91**, 528 (1964).
15. E. T. Mertz, O. E. Nelson, L. S. Bates, O. A. Veron, *Advance. Chem.* **57**, 228 (1966).
16. R. Bressani and E. T. Mertz, *Cereal Chem.* **35**, 227 (1958).
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Ribonuclease Activity in Normal and Opaque-2 Mutant Endosperm of Maize

Abstract. *Three strains of maize heterozygous for the* opaque-2 *mutant gene were self-pollinated to produce ears bearing both normal and opaque kernels. The ribonuclease activity of the* opaque-2 *endosperm was two to more than four times as high as the activity in the normal endosperm.*

The *opaque-2* mutant gene of maize drastically alters the amino acid composition of the endosperm proteins by greatly increasing the lysine content (*1*). The change comes through a replacement of zein by other proteins containing higher amounts of lysine. This finding is of great practical importance because the *opaque-2* seed possesses a higher food value than normal maize does (*2*). There is also a fundamental interest in the operation of a mutant gene that may act either by repressing the synthesis of a single storage protein or by preventing the normal development of the protein granules that are thought to be the site of zein (*3*).

Increasing ribonuclease activities are often associated with increasing concentrations of RNA in rapidly growing plant tissues (*4*). The soluble ribonuclease of maize, which liberates 2′,3′-cyclic nucleotides (*5*), is present in high concentrations in mature endosperm and reportedly increases during the development of the endosperm (*6*). The maximum activities of ribonuclease in endosperm range from about 500 units/g (fresh weight) up to about 1200 units/g for a number of hybrids, while activities of up to 1700 units/g were found in Illinois Low Protein, a strain with low amounts of zein (*7*).

The ribonuclease activities of normal and opaque endosperm were compared in three strains which were segregating for the *opaque-2* gene. The 07N strain was backcrossed three times, the others one time, after it was crossed with a source of the *opaque-2* gene. Then the self-pollinated plants were examined for ears containing both normal and opaque kernels soon after the time that a visible distinction could be made. Fifteen endosperms of each phenotype were separated from hulls and embryos. The endosperms were homogenized in ten times their weight of 0.05*M* sodium citrate buffer, *p*H 5.0, with 0.5*M* KCl, and the homogenates were then centrifuged to remove debris. Ribonuclease was assayed as described previously (*5*), with the units being defined in terms of the increase in the absorbancy at 260 mμ of acid-soluble material as RNA is digested. Dry weights were determined on a sample of ten kernels. The developing endosperms of the opaque kernels contained from 1.7 to 4.4 times as much enzyme per gram of fresh weight as did the normal endosperms from the same ear (Table 1). The lowest ribonuclease activity in the mutants was higher than any yet found in nonopaque kernels. The opaque endosperms had a higher water content at this stage of development than the normal endosperms did, so the differences are even greater when activities are based on units per dry weight. The dry weights of the opaque endosperms are less than those of the normals in all cases.

High activities of ribonuclease were also characteristic of the endosperm from plants homozygous for the *opaque-2* gene in backgrounds of five other strains as compared to the normal endosperm of the same strains. Large differences in activity were noticeable about a month after pollination. The differences between ribonuclease acitvities of normal and opaque endosperm are maintained even after the seed is put through normal drying procedures after harvest, but the activity may be considerably reduced.

The results of a few assays on lots of seed known to be heterozygous for the *opaque* gene and on individual normal-appearing seeds from one of the segregating ears assayed above suggest that there is no dosage effect of the *opaque-2* mutation on activities of ribonuclease; only the homozygous opaque kernels have the high concentration.

Preliminary experiments with disc polyacrylamide-gel electrophoresis (*8*) indicate that the ribonuclease in opaque endosperms is the same as that in normal endosperms.

The role of ribonucleases in normal development is unknown, and we may only speculate on the significance of the high activity in the *opaque-2* mutant. The enzyme might prevent zein synthesis by destroying a specific messenger RNA. However, its concentration in the normal endosperm is high enough to destroy all the RNA within 10 minutes, unless the enzyme is normally inhibited or separated from the RNA.

Table 1. Ribonuclease content of normal and opaque kernels from self-pollinated plants segregating for the *opaque-2* mutant gene. Each pair of assays was made on seed from a single ear.

Strain	Days after pollination	Ribonuclease (units/g*)		Dry weight (%)	
		Normal	Opaque	Normal	Opaque
07N	43	1390	2340	64	53.5
07N	43	1560	3540	66	58
Oh43	47	900	3600	73	69
Oh43	52	730	3250	68	59
Oh43	52	1380	3720	71.5	64
Oh45b	54	510	2110	73	66

* Fresh weight.

Endosperm ribonuclease has been purified, and its specific activity and amino acid composition are known (*9*). The high concentrations in opaque-2 endosperm do not materially contribute to the changed amino acid composition because ribonuclease contributes only 0.05 percent of the total protein. Ribonuclease contains 5.1 percent lysine.

The *opaque-2* mutation offers a unique opportunity for investigation of the possible function of ribonuclease in the control of protein synthesis in maize endosperm.

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References and Notes

1. E. T. Mertz, L. S. Bates, O. E. Nelson, *Science* **145**, 279 (1964).
2. E. T. Mertz, O. A. Veron, L. S. Bates, O. E. Nelson, *ibid.* **148**, 1741 (1965); E. T. Mertz and O. E. Nelson, Eds., *Proceedings of the High Lysine Corn Conference* (Corn Industries Research Foundation, Washington, D.C., 1966).
3. R. J. Dimler, in *Proc. High Lysine Corn Conf.* (**2**), p. 121.
4. L. Ledoux, J. Galand, R. Huart, *Biochim. Biophys. Acta* **55**, 97 (1962); J. Ingle and R. H. Hageman, *Plant Physiol.* **40**, 48 (1965).
5. C. M. Wilson, *Biochim. Biophys. Acta* **68**, 177 (1963); *ibid.* **76**, 324 (1963).
6. J. Ingle, D. Beitz, R. H. Hageman, *Plant Physiol.* **40**, 832 (1965).
7. C. M. Wilson, unpublished data.
8. B. J. Davis, *Ann. N.Y. Acad. Sci.* **121**, 404 (1964).
9. C. M. Wilson, *J. Biol. Chem.*, in press.

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Tetrodotoxin Blocks a Graded Sensory Response in the Eye of Limulus

Abstract. *The generalization that tetrodotoxin selectively blocks all-or-none electrical activity of nerve and muscle but has negligible effect upon graded responses of sensory systems does not appear to be valid for the* Limulus *eye. Tetrodotoxin reversibly blocks the graded transient component of this visual response, while the steady-state component of the response is relatively unaffected by the drug.*

The hazards of eating puffer fish have been well documented, and the relatively high concentrations of tetrodotoxin in these fish are more than adequate to account for the observed fatalities (*1*). Tetrodotoxin blocks the all-or-none type of electrical excitability of nerve and muscle (*2*). Experiments (*3*) on crustacean nerve and the giant axon of squid suggest that the drug specifically blocks the membrane processes associated with the initial transient conductance change of the neural impulse. On the other hand, it has been reported that tetrodotoxin has little or no effect upon the graded electrical response of several different sensory systems. The drug has been applied to the crustacean stretch receptor and the mammalian pacinian corpuscle (*4*), the mammalian cochlea (*5*), and several invertebrate sensory systems (*6*). In all cases tetrodotoxin blocked sensory nerve impulses, when observable, but had a relatively insignificant effect, if any, upon the graded sensory response. Data such as we now present indicate that tetrodotoxin has a pronounced effect on the graded visual response of the lateral eye of *Limulus*.

For our experiments the lateral eye of *Limulus* was excised, and a portion was mounted in a flow chamber. The preparation was exposed to a constant flow of sea water, and a glass micropipet electrode was inserted into a visual cell. The micropipet provided a salt bridge from the preparation to reversible electrodes and a d-c recording system. Tetrodotoxin was injected into the flow system through a fine capillary, and duration and rate of injection were controlled with a syringe drive. The test solutions were prepared by dissolving crystalline tetrodotoxin in normal sea water.

The eye was stimulated by a controlled flash program, and a constant stimulus program was maintained for all records of Figs. 1 and 2. In order to achieve a constant state of light adaptation, the eye had been stimulated for at least 1 hour before response 1 was recorded. When the eye was bathed in sea water alone, the visual response consisted of three components: (i) a short initial pulse, especially apparent in records 4 through 6, (ii) a transient component, and (iii) a steady-state component which was maintained for the duration of the stimulus. After application of tetrodotoxin, the transient component was markedly reduced in amplitude, while the amplitudes of the initial pulse and the steady-state component remained almost unchanged. The "noisy" character of these records deserves comment. Tetrodotoxin had been presented to this eye earlier, and its effects had been reversed by washing it out with sea water before the first record. After an eye had been exposed to tetrodotoxin, the responses often were "noisy" even when all other effects of the drug could be reversed in sea water.

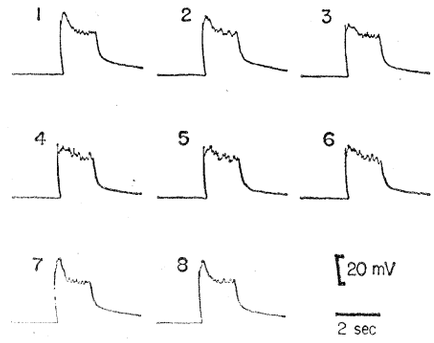

Fig. 1. Effect of tetrodotoxin on the graded response of the *Limulus* eye. Response 1 was a control recorded in sea water. Tetrodotoxin was injected into the bathing solution after record 1. Drug concentration, $< 10^{-5}$ g/ml. Pure sea water was used after record 4 to wash out the drug. All light flashes were of constant intensity and of 2-second duration, and they were repeated every 20 seconds. The curvilinear recording arc is defined for the voltage calibration.

In Fig. 2 response amplitudes are plotted against time, the data being derived from the records of Fig. 1. The transient component of the graded response is plotted in the upper curve,

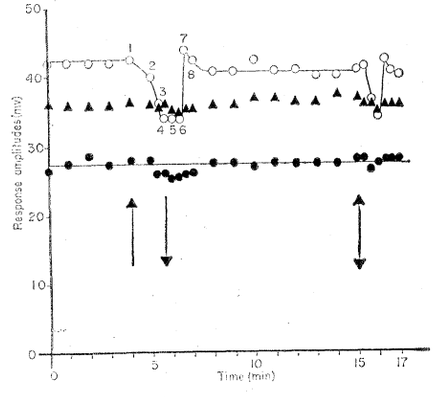

Fig. 2. A plot of response amplitude against time. The data were derived from responses which include the numbered records of Fig. 1. Open circles define the maximum amplitude of the transient component, the triangles define the peak amplitude of the initial pulse, and the solid circles define the amplitude of the steady-state component measured just before the stimulus was switched off. All potentials were measured relative to the resting level of the cell, which defines the zero value of the ordinate. Injection of tetrodotoxin is indicated by the upward arrow, addition of the sea-water wash was initiated at the downward arrow, and the double arrow indicates a brief injection of tetrodotoxin in the presence of the continuous sea-water wash. Drug concentration, $< 10^{-5}$ g/ml. The stimulus program was constant and as specified for Fig. 1.