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Ribonuclease Activity in Normal, opaque-2, and floury-2 Maize Endosperm during Development[1]

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ABSTRACT

The elevated ribonuclease activity produced in the endosperm of a maize (*Zea mays* L.) inbred, W64A, by homozygous opaque-2, results from a more than doubled rate of ribonuclease accumulation occurring prior to 16 days postpollination; after 16 days the rates in opaque-2 and normal are the same, suggesting that opaque-2 is no longer active. The pattern of ribonuclease increase in the opaque-2 dosage series indicates that opaque-2 is not fully recessive. Ribonuclease accumulation is not affected by floury-2 in a second inbred, B14. The results are discussed with reference to other proteins, notably zein, the net synthesis of which is affected by opaque-2.

Previously it was demonstrated that the RNase activity of the endosperm of an inbred line of maize homozygous for the mutant gene o_2 was much higher than corresponding normal (+) values throughout kernel development (2) and at maturity (2, 6). Twenty-three days after pollination the heterozygotes $o_2/o_2/+$ and $+/+/o_2$ had RNase activities about twice the normal (+/+/+) value, but only one-fifth that of $o_2/o_2/o_2$ (2). This report presents the results of a more detailed examination of the opaque-2 and normal RNase development curves, together with the early parts of the development curves of the opaque-2 and floury-2 dosage series. The mutant gene fl_2 is included because of its similarity to o_2 in producing extensive modifications of the amino acid composition of maize endosperm (4).

MATERIALS AND METHODS

The following lines of maize (*Zea mays* L.) were grown at Purdue University Agronomy Farm, Lafayette, Indiana in 1967. W64A, homozygous nonopaque (normal); W64A, homozygous opaque-2 (see Ref. 2); B14, homozygous nonfloury (normal); homozygous floury-2 back-crossed to B14 twice; homozygous opaque-2, homozygous floury-2 derived from a popcorn-dent corn cross and selfed twice. Ears were husked and frozen in Dry Ice in the field at intervals after pollination. Later, intact frozen kernels were removed, bulked according to age, mixed and stored at −20 C. The W64A material used to obtain the dosage series data of Figure 2 was from a planting seeded 2 weeks after the material used for Figure 1.

The preparation of enzyme extracts, conditions for the assay of RNase at pH 5.8, and definition of the unit of RNase activity have been described (2).

Regression techniques were employed to establish the equations for the regression of log (RNase units/endosperm) on days after pollination (Figs. 1 and 2).

RESULTS

The developmental curves for the RNase activity of W64A+ and W64Ao_2 endosperm from 5 to 60 days after pollination are shown in Figure 1. In both genotypes the RNase activity starts at about the same level and increases at an accelerating rate before entering an exponential phase. This exponential phase is attained at about 16 days postpollination in normal endosperm and is then maintained for a further 11 days. In contrast, o_2 has two exponential phases. The first (I in Fig. 1), occurring between 12 and 16 days postpollination, has a rate more than twice the maximum shown by normal endosperm. The second (II), between 17 and 22 days, shows a rate closely comparable with normal, as evidenced by the regression coefficients (Fig. 1 legend) which, when subjected to a t test (5), indicated that phase II and the exponential phase of the normal curve (from 16 to 28 days) have the same slope. Even following phase II the developmental curves of o_2 and normal continue to parallel each other, both virtually ceasing to increase at about the same time (day 40), and diverging only during the later stages of maturation when normal endosperm shows a decline in RNase activity not observed in o_2. The broken lines in Figure 1 show the curves obtained when RNase values of 10- and 15-day whole kernels are plotted, as in previous work (2), rather than the values for isolated endosperm.

Figure 2 shows the portion of the development curves of the opaque-2 dosage series lying between 13 and 24 days postpollination (13 and 25 for $o_2/o_2/o_2$). Since the slopes of the curves for $+/+/+$ and $+/+/o_2$ are very similar, whereas that for $o_2/o_2/+$ appears to be intermediate between $+/+/+$ (or $+/+/o_2$) and $o_2/o_2/o_2$, the data were examined statistically by a t test as described by Steel and Torrie (5). The regression coefficients for $+/+/+$ and $+/+/o_2$ (Fig. 2 legend) were not significantly different from each other at the 10% level but were significantly different from the regression coefficient for $o_2/o_2/+$ at the 5% and 1% levels, respectively. The regression coefficient for $o_2/o_2/+$ differed from the coefficient for $o_2/o_2/o_2$ at the 0.1% level of significance. As there is no reason to believe that these results could have arisen from purely physiological differences within the dosage series, it must be concluded that they strongly suggest that o_2 is not fully recessive for RNase, even though the opaque

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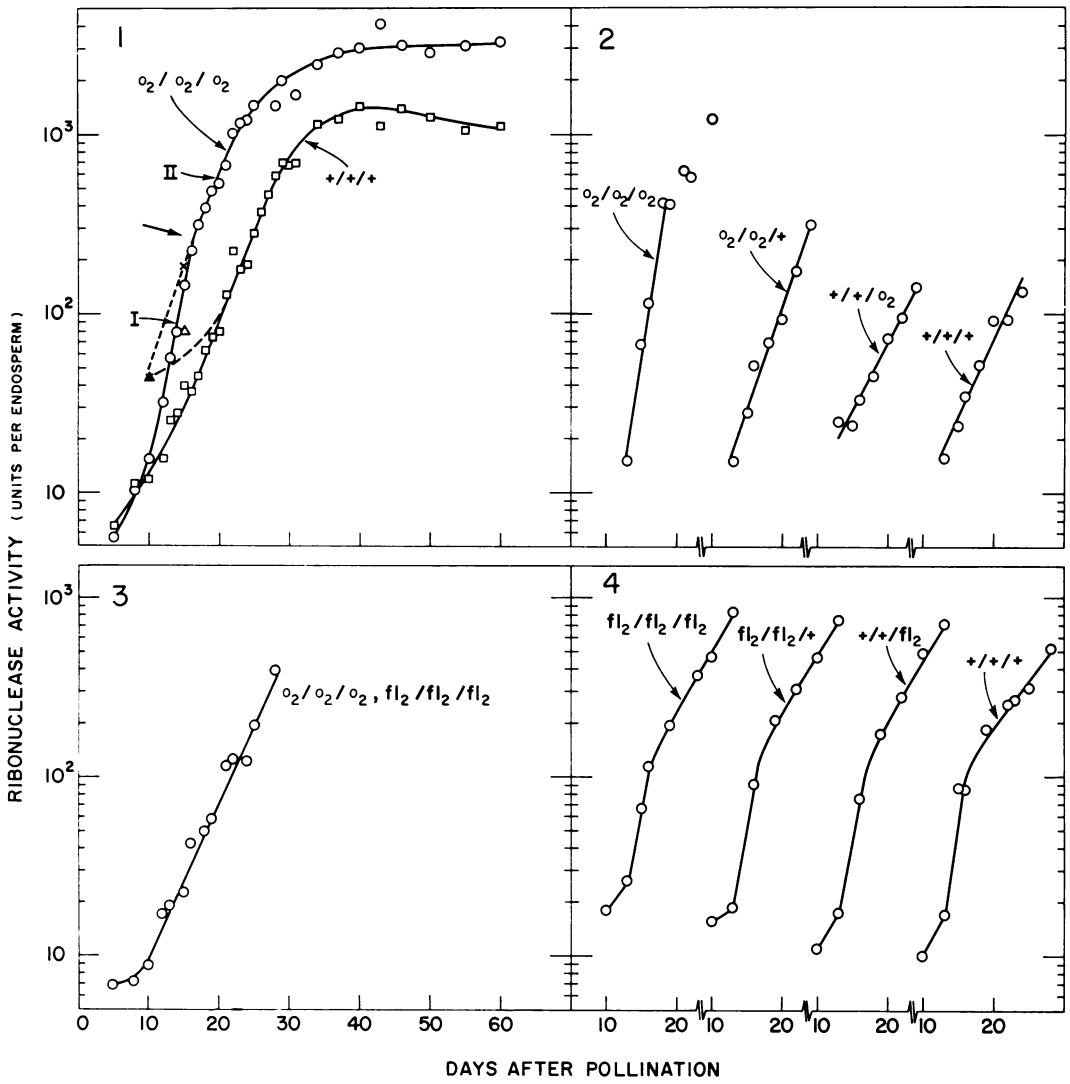

FIG. 1. Ribonuclease activity of normal (+/+/+) and opaque-2 ($o_2/o_2/o_2$) maize (W64A) endosperm during development. □—□: Normal endosperm; ○—○: opaque-2 endosperm; △- -△: normal whole kernels; ×- -×: opaque-2 whole kernels. Regression equations: opaque-2,I (12 through 16 days), log $\hat{Y}$ = 0.210 X − 1.00; opaque-2,II (17 through 23 days), log $\hat{Y}$ = 0.0969 X + 0.836; normal (16 through 28 days), og $\hat{Y}$ = 0.0982 X + 0.00916. Arrow indicates end of phase I and start of phase II.

FIG. 2. Ribonuclease activity of endosperm of opaque-2 dosage series (W64A) during early development. Regression equations: $o_2/o_2/o_2$, log $\hat{Y}$ = 0.249 X − 1.97; $o_2/o_2/+$, log $\hat{Y}$ = 0.113 X − 0.226; $+/+/o_2$, log $\hat{Y}$ = 0.0744 X + 0.348; +/+/+, log $\hat{Y}$ = 0.0852 X + 0.143.

FIG. 3. Ribonuclease activity of endosperm of floury-2 dosage series (B14) during early development.

FIG. 4. Ribonuclease activity of endosperm of double mutant opaque-2, floury-2 during early development.

phenotype is found only in homozygous o_2. The early developmental patterns of RNase activity in the floury-2 dosage series are shown in Figure 4. The differences between the members of the opaque-2 dosage series are not reproduced in the floury-2 series.

The early development curve of the double mutant, $o_2/o_2/o_2$; $fl_2/fl_2/fl_2$ is shown in Figure 3. The two phases observed with W64Ao_2 are not evident. The double mutant resembles W64A+ in showing a single, prolonged exponential phase extending late into development. As a suitable normal corresponding to the double mutant was not available, it was not established whether the presence of homozygous o_2 in the double mutant elevated the RNase level.

DISCUSSION

Previous work in this laboratory indicated that the primary reason for the higher RNase activity of o_2 over normal endosperm lay in the timing of the onset of the increase in RNase activity (2). Whereas in o_2 the increase appeared to be exponential from the first sampling (10 days), an apparent lag was shown by normal. It is now clear that this concept was in error and arose through the use of whole kernel RNase values for the 10- and 15-day postpollination samples in which the maternal tissue (testa, pericarp) made a considerable contribution to the total RNase activity. The broken curves of Figure 1 mimic the previous results very closely and demonstrate masking of the true endosperm RNase values. The use of isolated endosperm at early ages reveals that it is the initial rate of RNase increase (phase I), and not the timing, which ultimately produces the large differences. It should be noted that since RNase increases exponentially, the relatively small absolute difference between o_2 and normal at 16 days postpollination is all that is required to achieve the large absolute differences later in development.

At very early stages of development (<10 days) the tissue remaining after removal of the testa-pericarp is largely nucellus,

i.e., maternal tissue, with endosperm as a minor component. Therefore, differences in endosperm RNase activity are unlikely to appear. Extrapolations of phase I of the o_2 curve and the linear part of the normal curve (Fig. 1) intersect at about 9 days postpollination, but it is not possible to establish when the higher rate of RNase accumulation leading into phase I in o_2 is initiated.

The termination of phase I and the onset of phase II implies that a change in the mechanism controlling RNase increase occurs at 16 to 17 days postpollination. The subsequent close similarity in slope and shape of the o_2 and normal curves suggests that after 16 days the opaque-2 gene is no longer active, so that RNase activity increases during phase II at the same exponential rate as in normal endosperm.

This interpretation raises a question as to the generality of such a mechanism for other endosperm proteins the net synthesis of which is affected by o_2. The opaque-2 gene produces a marked reduction in the level of the zein complex of proteins in mature seed (3). An analysis of the development curve for zein (1) (Murphy, Dalby, and Mertz, manuscript in preparation) showed that in normal endosperm the zein content increased continuously from 10 days postpollination (the earliest sample examined). However, there was no increase of zein in o_2 until after 15 days postpollination, again pointing to a cessation of activity of o_2 after this time.

It is interesting to note that if the activity of opaque-2 is generally limited to the first 2 to 3 weeks following pollination, any procedure which would extend the period of opaque-2 activity beyond this time would be expected to reduce further, or even completely eliminate, zein from the endosperm. This should result in an even more radical change in amino acid composition and a further enhancement of the nutritional value of the protein of the endosperm over that already known to be associated with the presence of homozygous opaque-2.

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