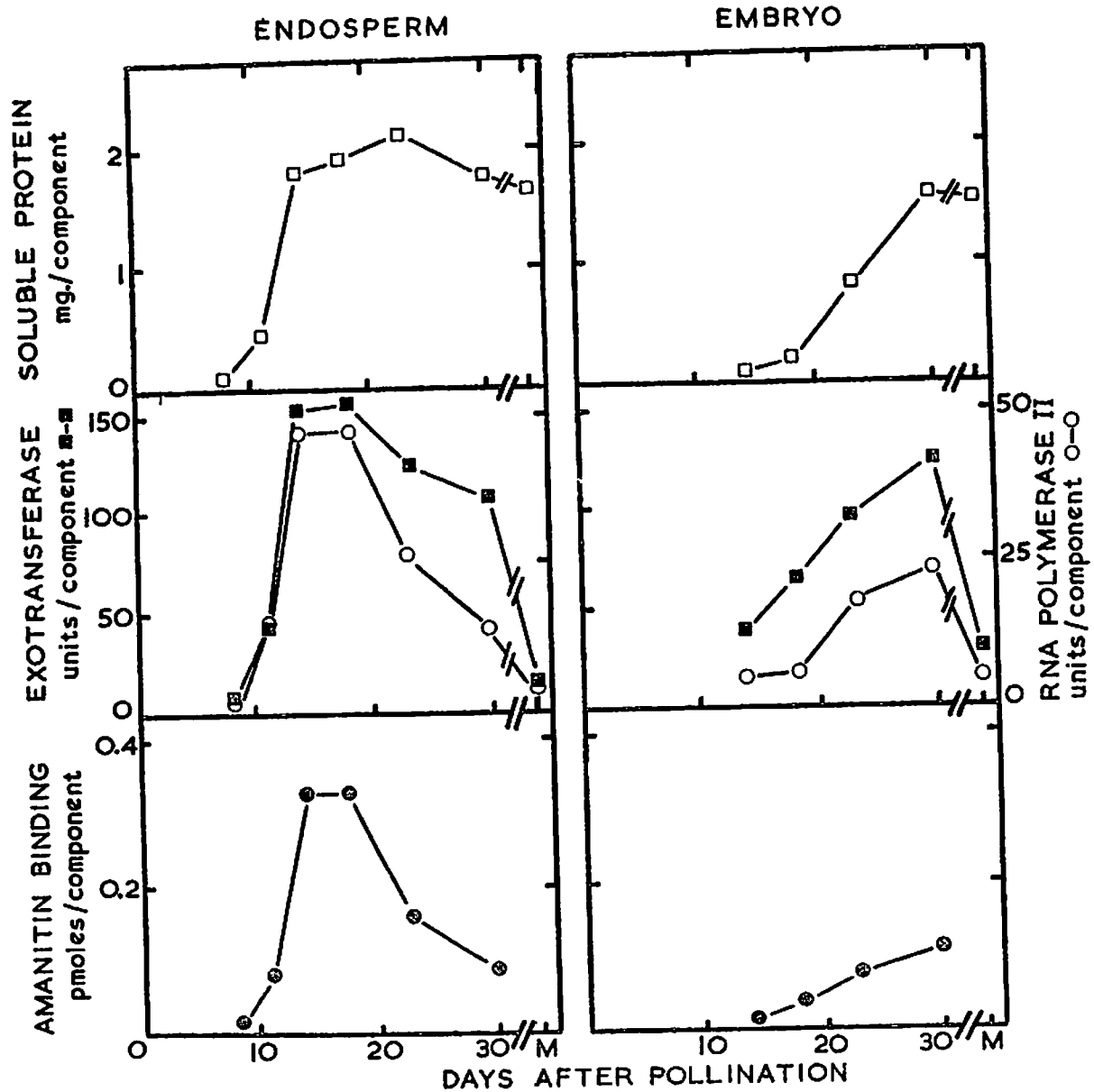


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Levels of RNA polymerase II and polynucleotide adenylyltransferase
in maturing maize kernels

Post-transcriptional modification of messenger RNA may regulate the expression of information derived from the chromosome. Most mRNAs are modified at the 3'-hydroxyl terminus by the addition of a poly (A) sequence. The ubiquity of this sequence and of enzymes capable of its synthesis suggest it plays a fundamental role in the expression of genetic information. Such a role could be direct as in the termination of transcription or indirect as in the processing of HnRNA or even more remotely as in the maintenance of mRNA function during translation. By examining tissues in different physiologic stages of maturation, we hope to find times when processing may be dissociated from transcription. As a first step we are determining if mRNA and poly (A) synthesis are directly coupled by following the two enzymic activities involved, namely RNA polymerase II and polynucleotide



adenyltransferase (exotransferase) during differentiation and development. The maize kernel was examined since it consists predominantly of two tissues: the endosperm, where mRNA synthesis may be specifically enhanced for the accumulation of starch synthesizing enzymes and storage protein, and the embryo, where the mRNA spectrum is likely to be more diverse among the many cell types differentiating during kernel development. Our approach has been to develop specific assays for these enzymes (Walter and Mans, 1975, Plant Physiol. in press). RNA polymerase II was determined as the α -amanitin sensitive incorporation of [^{14}C] UTP (1 enzyme unit is the incorporation of 1 mole UMP/min) or by a radioactive binding assay using [^3H] α -amanitin (Cochet-Meilhac and Chambon, 1974, Biochim. Biophys. Acta 353:160), a specific inhibitor of this enzyme. Exotransferase was assayed by the incorporation of [^{14}C] ATP into poly (A) (1 enzyme unit is the incorporation of 1 pmole AMP/min). Endosperms and embryos were dissected from maturing ears of corn (WF9 x Bear 38, waxy) beginning at 8 days after controlled self-pollination. Partially purified extracts were prepared by high speed centrifugation to remove cellular particulates, including DNA and ribosomes, and gel filtration to remove inhibitory low molecular weight components. Control experiments established that activity was not lost on dissection and that the sum of the observed activities in the isolated components corresponded quantitatively to the total activity in whole kernels.

Endosperms isolated at 8 days showed little activity (see figure), but a rapid increase in both activities was observed up to 14 days concomitant with the rapid proliferation of this tissue in the kernel. RNA polymerase II activity declined in the endosperm between 18 and 30 days. However, the loss of exotransferase activity was delayed and high levels of this enzymic activity were still present in the endosperm when the tissue was undergoing desiccation. The changes in RNA polymerase II were observed if the enzyme was assayed by *in vitro* RNA synthesis and by the binding of the specific inhibitor α -amanitin, indicating that levels of enzymic protein changed during maturation. Under our growth conditions, invertase activity (Tsai et al., 1970, Plant Physiol. 46:299) peaked at 14 days and ADP:glucose pyrophosphorylase (Ozbun et al., 1973, Plant Physiol. 51:1) reached almost maximal activity at 14 days in the endosperm. The coincidence of the increase in RNA polymerase and exotransferase activities with those of invertase and ADP:glucose pyrophosphorylase is consistent with the former's postulated role in providing mRNAs for the latter prior to the accumulation of starch and storage protein. Unlike the endosperm, the rise in embryo exotransferase preceded RNA polymerase activity and was proportionately higher during the 14 to 30 day period.

The changes in activities of the enzymes catalyzing RNA synthesis and polyadenylation of RNA differed in the two metabolically distinct tissues: (i) in the time of increased activities, (ii) in the relative level of activities attained and (iii) in the decay rates of the two activities and precludes a coupled relationship between them. A less direct and perhaps regulatory role for the exotransferase in processing transcripts is suggested.

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In vitro studies on the initiation of transcription by maize RNA polymerase II

The control of transcription in higher organisms is of primary importance in the regulation of gene expression. We have previously reported (Am. Soc. Agron. Abstr. 1975, p. 52) the construction of a model system for the study of transcription in maize. The *in vitro* system contains RNA polymerase II purified from maize seedlings as the transcriptive enzyme, while the allomorphic forms of bacteriophage ϕX174 provide homogeneous and genetically defined DNA templates. In our previous report, we demonstrated that circular, single-stranded DNA was