adenylyltransferase (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 [^{3}H] 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.

Trevor J. Walter and Rusty J. Mans

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

transcribed by the maize RNA polymerase without nicking the template, while linear strands of the same DNA (generated by DNAase treatment) were transcribed with the same efficiency. We concluded that transcription was initiated on internal sequences of the DNA molecule and not at nicks or ends.

In studies on mammalian RNA polymerases, it has been shown that nicking of double-stranded DNA (which facilitates local denaturation of the template) stimulated initiation by RNA polymerase (Meilhac and Chambon, 1973, Eur. J. Biochem. 35:454-463), while nicking of superhelical DNA (which removes the loosely base-paired regions of the superhelix) blocked initiation (Mendel and Chambon, 1974, Eur. J. Biochem. 41:367-378). In light of our findings with the maize enzyme, we suggest that in vitro initiation sites for eukaryotic RNA polymerases are always denatured or loosely base-paired regions of the DNA template and not nicks or ends of molecules.

Recently, we have found that the double-stranded, circular, superhelical DNA of ϕX174 replicative form I (RF 1) is a good template for transcription by maize RNA polymerase II, while intact, double-stranded DNAs that are not superhelical, such as phage T4 DNA, are poor templates. Similar observations have been made with mammalian RNA polymerases. Circular, superhelical DNAs are known to exist in chloroplasts, mitochondria, and nucleoli, and a significant proportion of chromosomal DNA may be superhelical as well. Perhaps DNAs must be superhelical in order to be transcribed in vivo and the loosely base-paired regions of the superhelix are the in vivo sites of RNA chain initiation.

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Further characterization of embryo ADP-glucose pyrophosphorylase

Endosperm adenosine diphosphoglucose pyrophosphorylase is of genetic and physiological interest because it is reduced in sh2 and bt2 mutants and it is an important and possibly regulatory enzyme in starch biosynthesis. Recent observations have suggested that the embryo and endosperm contain different forms of this enzyme. Preiss et al. (Pl. Physiol. 47:104, 1971) reported that the embryo enzyme differs from the endosperm enzyme in sensitivity to phosphate inhibition and extent of heat denaturation. It is not affected severely by mutants of sh2 and bt2 (Dickinson and Preiss, Pl. Physiol. 44:1058, 1969). These genetic and biochemical observations are, in general, reproducible (Hannah and Nelson, Pl. Physiol. 55:297, 1975). To further investigate the question of whether these tissues do contain different forms of this enzyme, a comparison of electrophoretic mobility was made.

Frozen 22-day-old kernels of the F_2 of W64A x 182E were dissected and the tissues were ground in phosphate buffer, passed through cheese cloth, centrifuged and the supernatant used as the enzyme source. Electrophoresis was done on 3.5% acrylamide-agarose gels as described in detail elsewhere (Hannah and Nelson, submitted to Biochemical Genetics). The gels permit rapid movement of pyrophosphorylase. It was found that the mobilities of the two enzymes were the same. This experiment was repeated approximately 10 times and in no case was a difference in mobility observed. Furthermore, Warren Bryce in Oliver Nelson's laboratory has obtained the same results. These results suggest that these tissues may contain the same form of pyrophosphorylase.

In cooperation with Drs. Trevor Walter and Rusty Mans, a more critical comparison, using highly purified preparations, of these two enzymatic activities is