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 4x174 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 F2 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
being conducted. It has been found that embryo preparations, purified as in previous experiments, contain a heat sensitive enzymatic activity which destroys a component of the reaction mixture, probably glucose-1-P. Thus, attention is now focused on the removal of this latter enzyme from the embryo pyrophosphorylase preparation in order to compare, in a definitive way, the pyrophosphorylases from the two tissues.

L. Curtis Hannah

Characterization of a gametophyte factor-like system on chromosome 5

A genetic system which leads to preferential fertilization by male gametes which contain \textit{bt-A} on chromosome 5 has been discovered and is now being characterized. Given below is a summary of some features of this system. Seen initially in Dr. Oliver Nelson's laboratory, this system has several features in common with gametophyte factors and, because of this, the gene located on chromosome 5 will be given the symbol \textit{Ga}. Because appropriate allelism tests with other known \textit{ga} loci on chromosome 5 have not been completed, the allele carried on the chromosome favored in fertilization will be denoted \textit{Ga*}-7001; the contrasting allele is termed \textit{ga*}-7001. Initially, it was found that plants of the genotype \textit{Bt/bt-A} gave rise to excess \textit{bt} kernels when self-pollinated or when used as male parents in crosses with \textit{bt-A/bt-A} plants. The reciprocal cross yielded the expected 50% frequency of \textit{bt} kernels.

Furthermore, this system appears to select male gametes which carry \textit{Ga*}-7001 for fertilization but not at the complete exclusion of \textit{ga*}-7001 gametes. This is suggested from the following lines of evidence. First, among 43 \textit{F}_2 families from the original \textit{F}_2 progeny, 42 contained \textit{bt} kernels at a frequency significantly greater than 25%. An average frequency of 39.5% was calculated from 17,570 kernels. If selection is complete for \textit{Ga*}-7001, this gene must be approximately 21 map units from the \textit{bt} locus. It follows then that 32% of the resulting \textit{F}_2 \textit{Bt/bt-A} kernels should be also \textit{Ga*}-7001/\textit{Ga*}-7001 and should give rise to 25% \textit{bt} in the \textit{F}_3 generation. Among 26 \textit{Bt/bt-A} plants selected from 4 \textit{F}_2 progenies, only one gave, upon self-pollination, a frequency not significantly greater than 25%. Again, 32%, or a total of 8, would be expected were the genetic distance equal to 21 map units. These latter results suggest, then, that \textit{Ga*}-7001 and \textit{bt-A} are much closer than 21 map units and this would suggest that some \textit{ga*}-7001 pollen also effect fertilization. Secondly, there appears to be genetic modification of the extent of \textit{Ga*}-7001 selection. This is from the following evidence.

Two \textit{F}_2 families contained \textit{bt} kernels at frequencies of 29.5% and 29.1%. These values are significantly greater than 25% but much lower than the overall average of 39.5%. Four \textit{Bt} kernels, selected from each of these families and which proved to be \textit{Bt/bt-A}, gave rise, upon self-pollination, to \textit{bt} kernels at average frequencies of 27.8% and 30.8% respectively. Similarly, \textit{Bt/bt-A} kernels selected from four other \textit{F}_2 progenies which had \textit{bt} frequencies of 53.8%, 39.5%, 49.5% and 40.7% produced \textit{F}_3 progenies in which the average percent \textit{bt} was 39.6%, 34.7%, 43.0% and 42.5%. The simplest explanation is that there exists a genetic system in which \textit{Ga*}-7001 gametes are favored in fertilization but that the extent of \textit{Ga*}-7001 selection is susceptible to genetic modification. Genetic heterogeneity for this modification existed in the original \textit{F}_1 progeny.

In analogy with other \textit{ga} systems, the simplest model to account for the observations thus far is that there exist two alleles of this locus: the allele \textit{Ga*}-7001, when carried in the female, favors fertilization by pollen also carrying \textit{Ga*}-7001. Data presented in Table 1 show, however, that this model cannot account for all the observations. It can be seen that plants heterozygous for other alleles of \textit{bt} yield, upon self-pollination, the expected results. However, when these plants are used as males onto \textit{Bt/bt-A} heterozygotes, an excess of \textit{bt} kernels is observed.