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.

Charles O. Gardner, Jr. and Rusty J. Mans

UNIVERSITY OF FLORIDA
Department of Vegetable Crops, Gainesville, Florida

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

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 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 Ga. Because appropriate allelism tests with other known ga loci on chromosome 5 have not been completed, the allele carried on the chromosome favored in fertilization will be denoted Ga*-7001; the contrasting allele is termed ga*-7001. Initially, it was found that plants of the genotype Bt/bt-A gave rise to excess bt kernels when self-pollinated or when used as male parents in crosses with bt-A/bt-A plants. The reciprocal cross yielded the expected 50% frequency of bt kernels.

Furthermore, this system appears to select male gametes which carry Ga*-7001 for fertilization but not at the complete exclusion of ga*-7001 gametes. This is suggested from the following lines of evidence. First, among 43 F_2 families from the original F_2 progeny, 42 contained 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 Ga*-7001, this gene must be approximately 21 map units from the bt locus. It follows then that 32% of the resulting F_2 Bt/bt-A kernels should be also Ga*-7001/Ga*-7001 and should give rise to 25% bt in the F_3 generation. Among 26 Bt/bt-A plants selected from 4 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 Ga*-7001 and bt-A are much closer than 21 map units and this would suggest that some ga*-7001 pollen also effect fertilization. Secondly, there appears to be genetic modification of the extent of Ga*-7001 selection. This is from the following evidence.

Two F_2 families contained 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 Bt kernels, selected from each of these families and which proved to be Bt/bt-A, gave rise, upon self-pollination, to bt kernels at average frequencies of 27.8% and 30.8% respectively. Similarly, Bt/bt-A kernels selected from four other F_2 progenies which had bt frequencies of 53.8%, 39.5%, 49.5% and 40.7% produced F_3 progenies in which the average percent bt was 39.6%, 34.7%, 43.0% and 42.5%. The simplest explanation is that there exists a genetic system in which Ga*-7001 gametes are favored in fertilization but that the extent of Ga*-7001 selection is susceptible to genetic modification. Genetic heterogeneity for this modification existed in the original F_1 progeny.

In analogy with other ga systems, the simplest model to account for the observations thus far is that there exist two alleles of this locus; the allele Ga*-7001, when carried in the female, favors fertilization by pollen also carrying 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 bt yield, upon self-pollination, the expected results. However, when these plants are used as males onto Bt/bt-A heterozygotes, an excess of bt kernels is observed.