Plant Molecular Biology Update

RFLP mapping of the \textit{abpl} locus in maize (\textit{Zea mays} L.)

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Received and accepted 12 June 1990

The plant hormone auxin controls a wide variety of growth and differentiation processes in higher plants. The molecular mechanism by which this control is exerted is essentially unknown. Auxin binds specifically to an auxin-binding protein (ABP) which is thought to be the first element in a signal transduction pathway [7]. However, the other components of an auxin-activated signal transduction pathway, which lead to the biological response, have not been identified. If mutations in the presumed signal transduction pathway could be obtained, they would be extremely useful in elucidating the molecular mechanism of auxin action.

Auxin biosynthesis and auxin response mutants have been described in some dicotyledonous plants [6, 9]. That such mutants are found at frequencies 10–100 times lower than null mutants of other loci [3, 6], e.g. starch or lipid biosynthesis [6], strongly suggests that null mutants related to auxin physiology are lethal [8]. The mutants that have been characterized show pleiotropic effects and hence a variety of phenotypes. By determining the genetic locus of at least one component of the auxin signal transduction pathway, we should be able to characterize auxin response phenotypes better. This will allow us to look for similar phenotypes with the goal of finding mutants in the signal transduction pathway. We have used RFLP mapping (restriction fragment length polymorphism mapping) to assign a locus to the ABP, the putative first element in the auxin signal transduction pathway.

Maize recombinant inbred lines from the F2 populations of TXCM as well as the parental lines T232 and CM37 [1] were used to prepare genomic DNA from leaves. The isolation of an ABP cDNA clone (pBS.\textit{ABP}) is described elsewhere [10]. All restriction enzymes and the nick translation kit were purchased from Bethesda Research Laboratory, Bethesda, MD. DNA was extracted from young leaves (approx. 1 g of tissue) following a procedure of Cone [2] with some modifications. In brief, leaf tissue frozen on dry ice at the time of harvest was homogenized in liquid nitrogen with pestle and mortar. The resulting fine powder was added to 5 ml homogenization buffer (7 M urea, 0.35 M NaCl, 0.05 M EDTA, 0.01 M Tris-HCl pH 7.6, 2% sodium lauroylsarcosine) in a glass scintillation vial and rocked on a shaker until it appeared homogeneous (ca 15 min). An equal volume of phenol, equilibrated with homogenization buffer, was added and again shaken until apparent homogeneity (ca 15 min). The highly viscous slurry was centrifuged and the aqueous phase was extracted with an equal volume of chloroform : isooamyl alcohol (24 : 1). DNA was precipitated by adding sodium acetate (final concentration 0.2 M) and 0.55 volumes of isopropanol. High molecular weight DNA was spooled out with a bent pasteur pipette and resuspended in 4 ml TE (10 mM Tris-HCl pH 8, 1 mM EDTA). A final ethanol precipitation in the presence of 2 M ammonium acetate yielded a nucleic acid preparation that could be digested with restriction enzymes.

Restriction digests were performed according to manufacturer's protocols using 1.5 \( \mu \)g of DNA and 20 units of enzyme for a 3 h incubation time at the appropriate temperature. Restriction fragments were separated on 0.8% agarose gels and blotted onto nitrocellulose (Schleicher & Schuell).
Fig. 1. Southern blot of maize genomic DNA from the parental lines T232 and CM37 indicated by T and CM respectively. The restriction enzymes used are indicated above the lanes. Nick translated ABP cDNA was used as a hybridization probe.

Fig. 2. Southern blot of maize genomic Ssr I DNA fragments from the recombinant inbred F2 families (TXCM) and the parental lines T232 and CM37. Nick translated ABP cDNA was used as a hybridization probe. Note that the observed hybridization pattern of the recombinant inbreds matches either one of the parental lines (T232 or CM37).