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Molecular cloning of the *a1* locus of *Zea mays* using the transposable elements *En* and *Mu1*

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The *a1* locus of *Zea mays* has been cloned using transposable elements as gene tags. The strategy was to make genomic libraries from maize stocks with *a1* mutations induced either by *En(Spm)* or by Robertson's *Mutator*-system. These libraries were then screened with either *Spm-I8* and *En1*, for the *En*-containing mutant, or with *Mu1* for the *Mu*-induced mutation. There are many *En* and *Mu1* hybridizing sequences present in the maize genome, however, by a process of cross-screening of the positives from the two libraries and by molecular analysis of the *En*-positive clones it was possible to identify clones in both libraries carrying all or part of the *a1* gene.

Key words: a1 locus/gene tagging/transposable elements

Introduction

The *a1* locus of *Zea mays* has been extensively used in the study of transposable elements (for recent reviews, see Nevers *et al.*, 1985; Fedoroff, 1983). Many different transposable element systems have been studied at the *a1* locus and in particular it has been used to study genetically many features of the *En(Spm)* system. The product of the *a1* gene is involved in the anthocyanin biosynthetic pathway (Kirby and Styles, 1970). Recessive mutations lead to a colorless aleurone layer while the somatic instability characteristic of transposable element-induced mutations is usually expressed as colored sectors on a pale or colorless background. Isolation of a molecular clone carrying the *a1* gene would allow molecular analysis of the transposable elements present at the locus. However the product of the gene has not been identified and therefore it is not possible to clone the locus using classical recombinant DNA techniques. It was decided to utilise the presence of transposable elements at *a1* in the isolation of the gene. Such an approach was first shown to be feasible in maize when the *Ac* element was used to isolate the *bz1* locus (Fedoroff *et al.*, 1984). This experiment exploited the fact that an internal portion of the *Ac* element is present in low copy number in the maize genome and thus could be used as a probe in the isolation of the gene. This strategy could not be used to isolate the *a1* gene as the elements present at the locus, for which molecular probes were available, are all present in many copies in the genome. It was therefore decided to adopt the strategy of making genomic libraries from two lines of maize with different element present at *a1*. It was postulated that having initially selected clones using the transposable element probes it would be possible to cross-hybridize the clones from the two libraries with the hope that any cross-hybridizing clone would be a candidate for the *a1* gene. Using this strategy we have isolated clones carrying all or part of the *a1* gene.

Results

Isolation of Mu1 homologous clones from the a1-Mum2 stock

The two elements chosen for use as molecular probes were *Mu1* and *Spm-I8*. The 1.4-kb *Mu1* element is thought to be responsible for many of the mutations obtained in experiments with Robertson's *Mutator* line (Robertson, 1978; Bennetzen *et al.*, 1984; Freeling, 1984) and a number of stocks with mutations at the *a1* locus in the *Mutator* line were available. The *Mutator*-induced unstable *a1* mutant used in cloning in this work was *a1-Mum2*.

DNA isolated from the homozygote *a1-Mum2* stock was digested to completion with *Eco*RI and cloned into λgtWES.λB. Approximately 2 x 10^6 plaques were obtained and screened by plaque hybridization with the *Mu1* probe (see Materials and methods). Approximately 150 positive clones were isolated. Fifty of these positives were analyzed at the DNA level. By restriction analysis and re-screening of all the recombinants at the phage level with subclones of unique fragments of some of the isolated clones the final number of possibly different *Mu1* homologous clones was 35. The enzymes *Taq*I and *Mlu*I both have sites within the inverted repeats of *Mu1* and yield fragments of 1026 bp and 958 bp, respectively (Bennetzen *et al.*, 1984). Digestion of the 35 *Mu1* homologous clones from *a1-Mum2* with *Taq*I and *Mlu*I indicated that all contained the expected fragments and at this level are identical to *Mu1*.

Analysis of the Spm-I8 homologous clones of the a1-m(papu) stock

Spm-I8 is the 2.2-kb receptor element of the *En(Spm)* system isolated from the *wx-m8* allele (Schwarz-Sommer *et al.*, 1984). Many stocks were available containing either the receptor component (*I*) or the autonomous element (*En*) at the *a1* gene. The *a1-m(papu)* allele (Peterson, 1970, 1981; Nowick and Peterson, 1980, 1981), which contains an autonomous *En* at the *a1* locus, was used in the cloning experiment using *Spm-I8* as probe. It was hoped that the *En1* system would resemble the *Ac/Ds* system in that receptor elements are most often deletion derivatives of the autonomous element (Döring and Starlinger, 1984; Fedoroff *et al.*, 1983) and that therefore if the receptor element *Spm-I8* was used as probe, clones carrying any homologous sequences, including the autonomous *En* element, would be selected. The process of screening the clones isolated in these experiments was greatly simplified when the autonomous *En* element, designated *En1*, at the *wx* locus was isolated (Pereira *et al.*, 1985). The presence of internal restriction fragments in this element which are not present in *Spm-I8* allowed the further screening of the

I homologous clones for those having an internal structure similar to *En1*.

Plant DNA isolated from a stock of maize homozygous for the *a1-m(papu)* allele was partially digested with *Mbo*I, size fractionated and fragments in the size range 14 – 25 kb were cloned into the *Bam*HI site of the λEMBL4 vector. Approximately 250 000 recombinant plaques were screened by plaque hybridization with a 2.5-kb *Sal*I fragment containing the *Spm-I8* element. From the *a1-m(papu)* library, 50 recombinant clones were isolated which hybridized to *Spm-I8*. At this time the clone of the autonomous *En* element, *En1*, became available. This autonomous *En* element from the *wx-844* allele is 8.4 kb in size and contains two internal *Eco*RI fragments of 3.0 kb and 1.4 kb in size which do not hybridize to *Spm-I8* (Pereira *et al.*, 1985). It was thought likely that the autonomous *En* element at the *a1* locus in the *a1-m(papu)* allele would have the same molecular structure as *En1*. Therefore the two internal *Eco*RI fragments of *En1* were used as probes to screen the 50 *Spm-I8* positive clones. Thirteen clones hybridized to a mixture of both fragments. The hybridization was then repeated to these 13 clones using the 3.0-kb and 1.4-kb probes independently. Eight clones hybridized to both fragments and six of these eight hybridized equally strongly to both probes and these were chosen for further analysis.

DNA was isolated from each of these '*En*'-positive clones and compared by *Eco*RI restriction pattern to the *En1*-containing phage clone wx-844-148. Only one of the six '*En*' positives contained both the 3.0-kb and 1.4-kb *Eco*RI fragments. This clone has been called c110En. Of the other five '*En*'-positives, none contained either the 3.0-kb or the 1.4-kb fragment intact.

Detailed restriction enzyme comparison of the *En* insert of c110En with *En1* has not been carried out, with the exception of the initial analysis with *Eco*RI which identified c110En. However heteroduplex analysis of total phage from wx-844-148 and c110En reveals a double-stranded region of 8.1 kb (Figure 1) which is in good agreement with the size of 8.4 kb determined for *En1*. This result, together with *Eco*RI restriction data, indicates that the *En* element in c110En is structurally identical with the autonomous element *En1* within the limits of discrimination of the heteroduplex analysis. This is a strong indication that c110En represents the *En* element present at the *a1* locus in the *a1-m(papu)* stock as there is only one active *En* element present in this strain.

Cross-hybridization of the 'En' clones with Mu clones

The next step was to test whether sequences present on any of the six *En* clones, which showed homology to the two internal *Eco*RI fragments of *En1*, were also represented in the 35 *Mu1*-positive clones. It was predicted that the only clones which would cross-hybridize between the two libraries would be those carrying the elements at the *a1* locus (or those carrying common repetitive DNA sequences). For this experiment phage DNA from the 35 *Mu1*-positive clones was digested with *Eco*RI and hybridized with a mixture of nick-translated DNA from the *En*-positive clones. c10Mu was the only clone which hybridized strongly to the mixture of probes but six other clones showed weak hybridization. These seven clones were then hybridized with each of the *En* clones individually and c10Mu was the only clone which hybridized, and it hybridized only with c110En (Figure 2). The fact that the only *En* positives which hybridized with any of the *Mu1* positives is the same clone which has an *En* structure identical to the autonomous element *En1* is very strong support in favour of c110En and c10Mu being clones which together contain all or part of the *a1* gene. A summary of the pathway by which the c100En and c10Mu clones were identified is given in Figure 3.

Restriction mapping and heteroduplex analysis of c10Mu and c110En

The c10Mu clone contains a 7.8-kb *Eco*RI insert in λ gtWES. This fragment has been subcloned in pUC9 and a restriction map of the fragment has been constructed (Figure 4). The position of

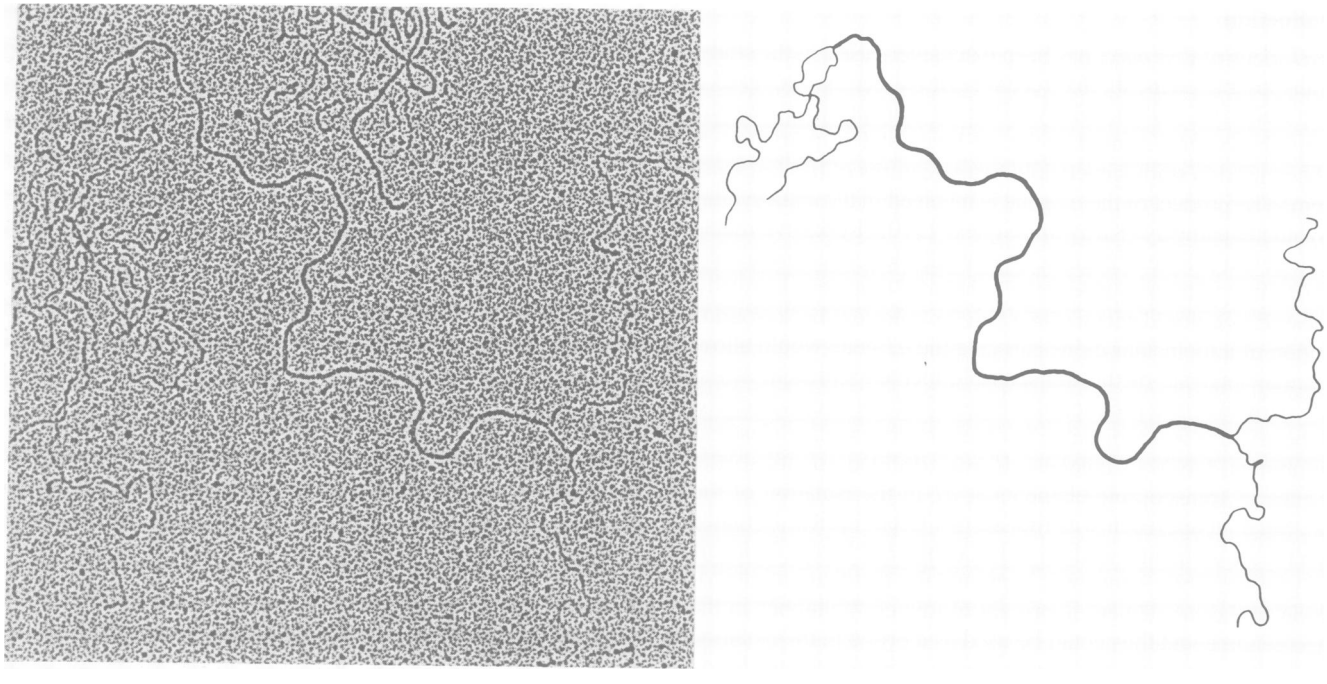

Fig. 1. Electron micrograph and line drawing of the heteroduplex formed between two phage DNA from c100En and the *En1*-containing clone wx-844-148. The length of homology between the two clones is 8.16 kb based on the measurement of 12 molecules. This is in good agreement with the size of 8.4 kb calculated for the *En1* element (Pereira *et al.*, 1985). The *En* inserts in the two clones are in opposite orientation relative to the phage arms and therefore no hybridization between the phage DNA is seen in this molecule.

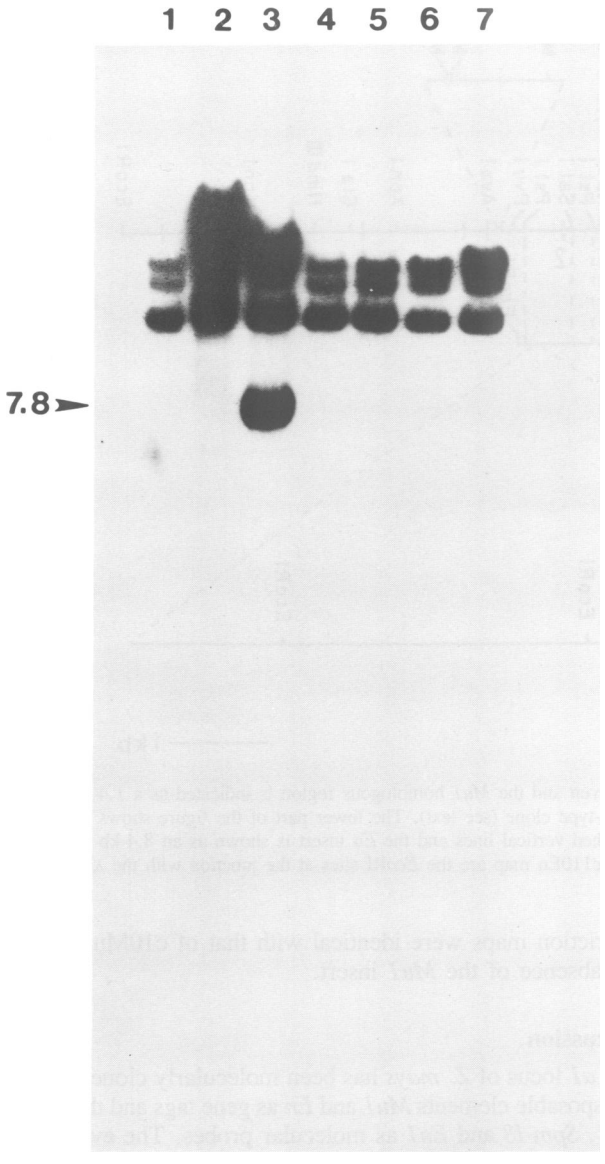

Fig. 2. Hybridization of seven *Mu1* homologous clones with c110En. Phage DNA from the *Mu* clones was digested with *Eco*RI and hybridized with total phage DNA from c110En. The only insert specific hybridization seen is the the 7.8-kb insert of c10Mu (**lane 3**).

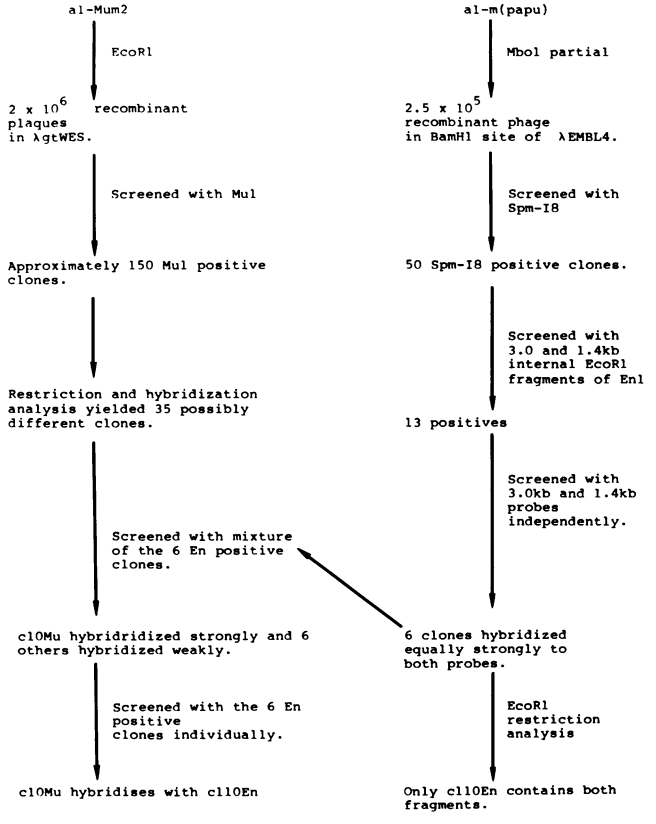

Fig. 3. This figure summarizes the pathway by which the *a1*-containing clones c10Mu and c110En were identified.

the *Mu1* homologous region within this fragment was determined by hybridization with the *Mu1* probe and by the position of the *Mlu*I restriction sites which are present in the inverted repeats of *Mu1* (Barker *et al.*, 1984). The position of the element is indicated in Figure 4 as a 1.4-kb insert in a 6.4-kb *Eco*RI fragment.

To determine which region of c110En hybridizes with c10Mu, phage DNA of c100En was digested with *Eco*RI and hybridized with the c10Mu 7.8-kb insert. The result of this hybridization is shown in Figure 5a. Two fragments of c100En, the 4.6-kb and the 1.7-kb fragments, hybridize with the c10Mu clone. These are also the fragments which hybridize to *Spm-I8* (Figure 5b) and are therefore considered to be the junction fragments between the *En* element of the *a1-m(papu)* allele and the *a1* gene. In Figure 5c and d the hybridization of the internal 3.0-kb and 1.4-kb *Eco*RI fragments of *En1* to the *En1*-containing clone wx-844-148 and to the c100En clone is shown which indicates that c110En contains the same internal *Eco*RI fragments as wx-844-148. Restriction mapping, hybridization studies and comparison with the *Spm-I8* and *En1* maps has allowed the alignment of the 4.6-kb and 1.7-kb fragments with the c10Mu map which is shown in Figure 4. The 4.6-kb fragment of c100En is an internal fragment which contains a 2.3-kb region of homology to c10Mu as indicated in Figure 4. Analysis of the 1.7-kb *Eco*RI fragment, in which one *Eco*RI site represents the junction with the EMBL4 vector, indicates that it contains a very short region of homology, of ~150 bp, to c10Mu which is supported by the fact that the hybridization of this fragment to c10Mu is weaker than the hybridization with *Spm-I8* (Figure 5a and b). The short region of homology includes a *Pst*I site (Figure 4) which is consistent with the *En* element in c110En being inserted in the 0.6-kb *Pst*I fragment of wild-type and with the point of insertion being very close to the *Pst*I site at the right end of the 0.6-kb *Pst*I fragment as shown in Figure 4.

Heteroduplex analysis confirms the data obtained by the restriction analysis (Figure 6). A heteroduplex between total phage DNA from c110En and the 7.8-kb insert of c10Mu shows a double-stranded region of 2.4 kb which can be aligned relative to the restriction map by its distance from the characteristic stem-loop structure of *Mu*. The distance between the end of the double-stranded region and the *Mu* stem-loop is estimated to be 400 bases which is in good agreement with the distance as calculated from the restriction maps. A large loop structure representing the *En* insert of c110En was not observed since the length of homology between c110En and c10Mu at the right side of the c100En clone as depicted in Figure 4 is not long enough to generate a heteroduplex.

Isolation of c10Mu homologous clones from a1 wild-type stocks

The representation of the c100En and c10Mu clones as shown in Figure 4 has been confirmed by cloning the homologous

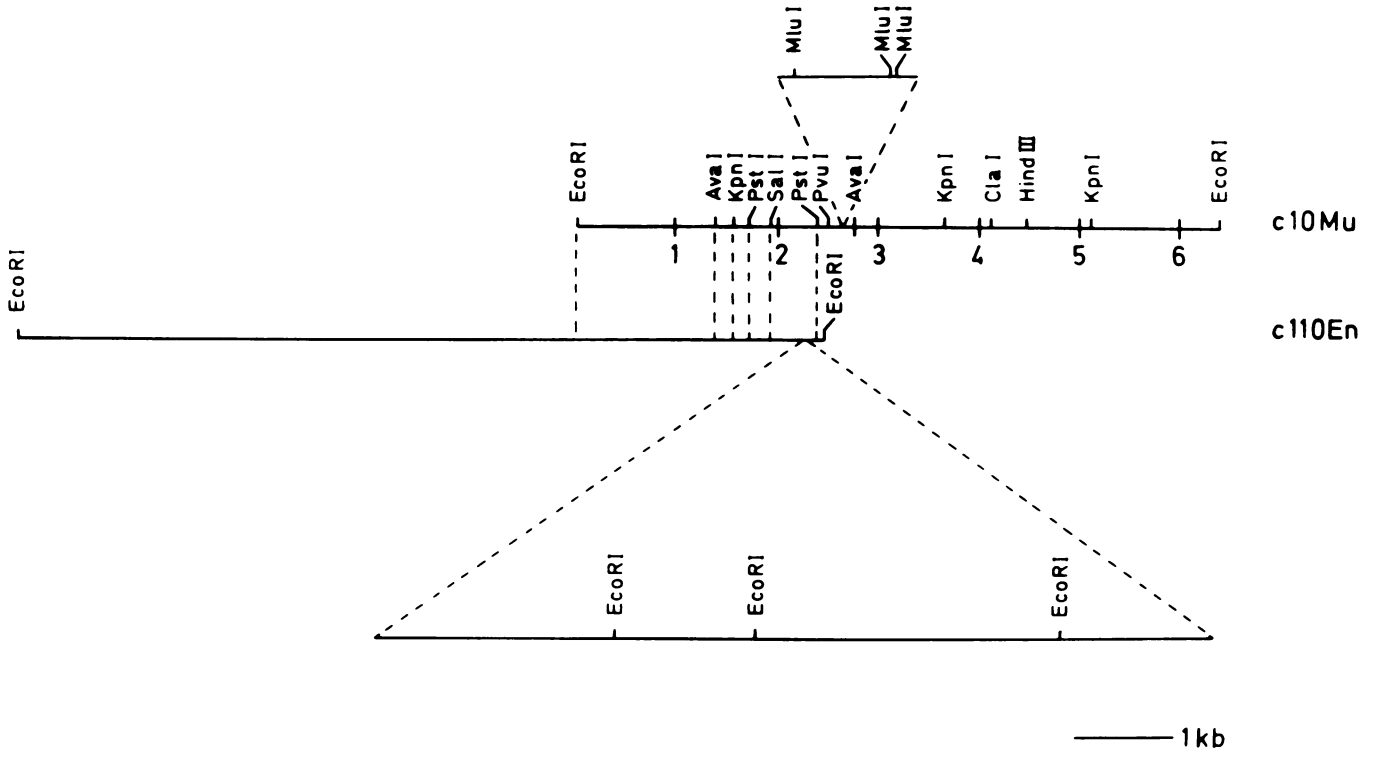

Fig. 4. In the upper part the restriction map of the 7.8-kb *Eco*RI insert of c10Mu is given and the *Mu1* homologous region is indicated as a 1.4-kb insert in a 6.4-kb *Eco*RI fragment. This representation has been confirmed by analysis of the wild-type clone (see text). The lower part of the figure shows the alignment of the c110En clone with the c10Mu map. Restriction sites shared are indicated by dashed vertical lines and the *En* insert is shown as an 8.4-kb insertion close to the *Pst*I site at 2.4 kb on the c10Mu map. The *Eco*RI sites at the ends of the c110En map are the *Eco*RI sites at the junction with the λEMBL4 vector.

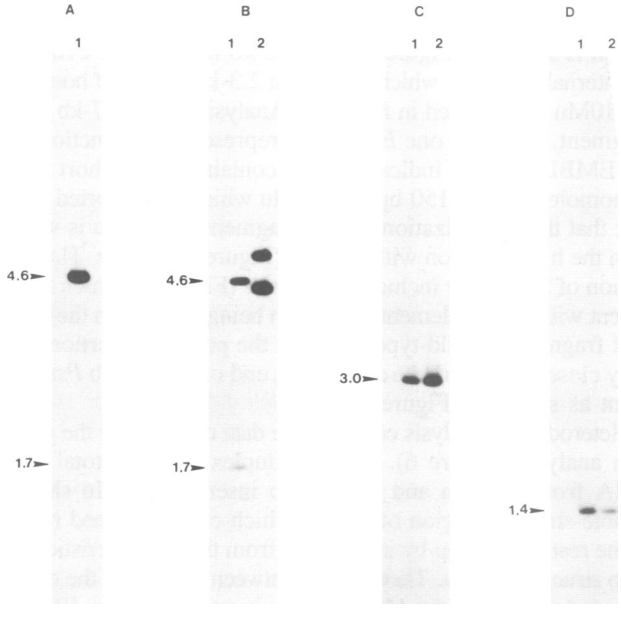

Fig. 5. Hybridization of phage DNA from c110En (**lane 1**) and wx-844-148 (**lane 2**) with c10Mu (**A**), *Spm-I8* (**B**, 3.0-kb *En1 Eco*RI fragment (**C**) and 1.4-kb *En1 Eco*RI fragment (**D**).

fragments from two stocks of maize with a wild-type *a1* phenotype. The two stocks used were Line C and Robertson's *Mutator* line, which is the progenitor of *a1-Mum2* allele. The probe used in cloning was the 0.6-kb *Eco*RI fragment of c10Mu, which appears to be unique in the maize genome. From both lines the clones isolated contained a 6.4-kb *Eco*RI fragment whose restriction maps were identical with that of c10Mu except for the absence of the *Mu1* insert.

Discussion

The *a1* locus of *Z. mays* has been molecularly cloned using the transposable elements *Mu1* and *En* as gene tags and the elements *Mu1*, *Spm-I8* and *En1* as molecular probes. The evidence that the molecular clones isolated from these experiments do indeed carry *a1* gene sequences is as follows.

(i) In the library from the *a1-m(papu)* allele, which has been shown genetically to carry an autonomous *En* element at the *a1* locus (Peterson, 1970, 1981; Nowick and Peterson, 1980, 1981), only one clone, c110En, was isolated which had an internal molecular structure indistinguishable from that of the autonomous *En* element *En1* (Pereira *et al.*, 1985). This in itself is a strong indication that this clone represents the autonomous *En* element present at the *a1* locus in the *a1-m(papu)* allele.

(ii) A library was also constructed from a maize stock with a mutation at the *a1* locus induced in Robertson's *Mutator* line (Robertson, 1978). Many if not all mutations in Robertson's *Mutator* line are due to the insertion of the 1.4-kb *Mu1* element (Bennetzen *et al.*, 1984; Freeling, 1984). Of 35 different *Mu1*-positive clones only one, c10Mu, contained sequences which hybridized to any of the clones selected in the *a1-m(papu)* library and this clone hybridized only with c110En, the clone containing the *En1*-like element.

(iii) The region homologous to the c10Mu clone has been cloned from the *Mu* progenitor line and also from the inbred Line C, both of which are wild-type with reference to the *a1* locus. Clones isolated from both lines contain 6.4-kb *Eco*RI fragments, which is the predicted size at least in the *Mu* progenitor line for

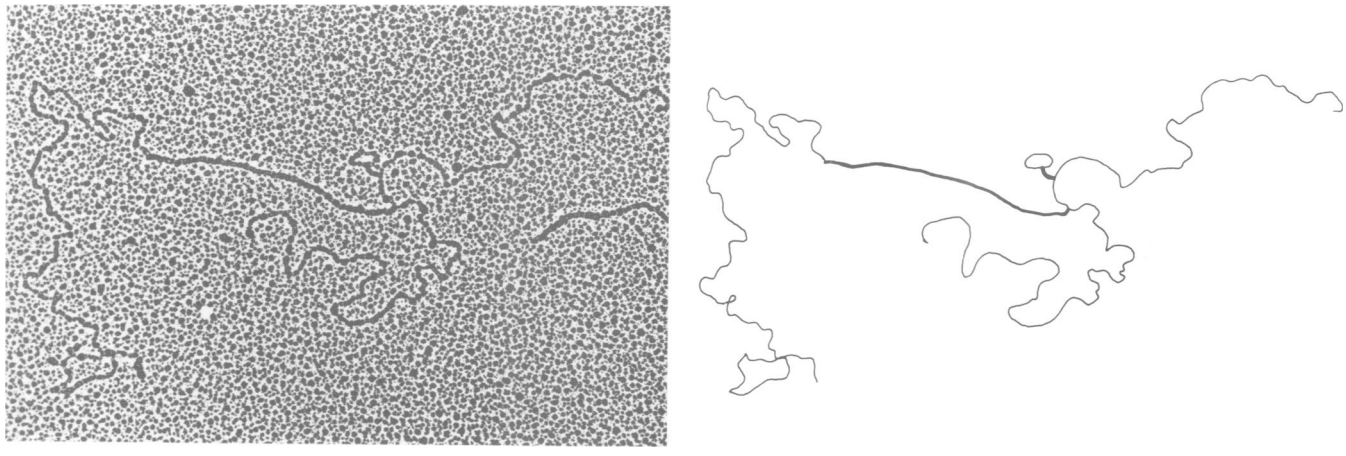

Fig. 6. The electron micrograph and line drawing of the hetroduplex formed between the 7.8-kb insert of c10Mu and total phage DNA from c110En. A double-stranded region of 2.3 kb is seen. The stem-loop structure of the *Mu1* homologous element is also observed.

the wild-type allele. The restriction map of this 6.4-kb fragment is consistent with this sequence representing the wild type allele.

Further evidence that the clones represent *a1* gene sequences comes from recent work in which the 0.6-kb *Pst*I fragment of c10Mu was used to probe genomic libraries from two maize strains containing different states of the *a1-m1* allele (Schwarz-Sommer *et al.*, unpublished). The mutation in this allele is due to the presence of an *Spm* receptor element at the *a1* locus and is of interest due to the many phenotypic variants (termed 'states of the element' by McClintock) which have been isolated from the original *a1-m1* allele (McClintock, 1955, 1957, 1958; Reddy and Peterson, 1984). From the genomic libraries probed initially with the 0.6-kb *Pst*I fragment of the c10Mu clone, clones were identified which contained sequences homologous to the *Spm-I8* element. These clones, which are considered to be clones of the *a1-m1* alleles, are at present being analyzed.

The *a1-m(papu)* allele containing the autonomous *En* element at the *a1* locus was isolated as a phenotype variant of the *a-m(dense)* allele, which also contained an autonomous *En* at *a1* (Peterson, 1961, 1970, 1981; Nowick and Peterson, 1980). Other variants of the original *a-m(dense)* allele are available and it will be of interest to study the molecular basis of this variation. It will also be interesting to study the *a1-m2* allele which contains an autonomous Spm element whose phenotype is very different from the other *En(Spm)*-induced mutations at the *a1* locus (McClintock, 1951, 1961, 1962).

The fact that the *a1-Mum2* allele contains a *Mu1*-like element at the *a1* locus lends further support to the theory that *Mu1* is the causative agent of most mutations in Robertson's *Mutator* line. Analysis of two other unstable *a1* mutations induced in the *Mu* line is at present being carried out, as is the analysis of three stable *a1* mutations induced in the same line.

The availability of the molecular clone carrying the *a1* gene will allow all these events to be studied at the molecular level and will also allow the study of other elements at the locus. Of these the receptor component, *ruq*, of the *Uq/ruq* system is of particular interest (Friedemann and Peterson, 1982).

Finally the cloning of the *a1* locus using transposable elements as gene tags demonstrates the power of this technique in the isolation of genes for which the gene products have not been identified. It is foreseeable that such techniques can be used to isolate any gene where an unstable phenotype can be easily identified. By genetic means it should be possible to isolate transposable element induced mutations at such loci and then clone the mutant alleles using the transposable element probes. The ease of identification of the desired clone is somewhat dependent on the copy number of the probe within the genome. The *Ac* element, for example, has an internal region which is present in low copy number in the maize genome and this segment was used as a probe to isolate the *bz1* locus (Fedoroff *et al.*, 1984). The *Mu1* and *En* elements used in this study both are present in many copies (30 – 50) in the genome. However it was possible by the consecutive use of different parts of the *En* element as probe, to identify a clone carrying the complete element and there was only one such clone in the library. This type of analysis was not possible for the *Mu1* clones. However, the availability of the *Mu1* library greatly simplified the identification of the *a1*-containing clone. Such cross-screening of libraries is useful when the only mutants available at the desired locus are due to elements with a high copy number, resulting in a large number of clones to be analyzed.

Materials and methods

Plant strains

The *a1-Mum2* strain containing the *Mu* (Robertson, 1978) induced mutation at the *a1* locus was isolated from a cross in which the Pl aleurone *Mu* stock was used as male parent and the homozygous recessive *a1 sh2* was used as female.

The *a1-m(papu)* containing an autonomous *En* element at the *a1* locus was first described by Peterson (1970) as a phenotypic variant of the *a-m(dense)* allele (Peterson, 1961).

Line C used as a source of the wild-type *a1* allele is a color converted W22 maize line developed by Dr. R.A.Brink, University of Wisconsin.

Isolation of plant, bacteriophage and plasmid DNA

Plant DNA was isolated from leaf material as described by Gupta *et al.* (1983) or Schwarz-Sommer *et al.* (1984). Isolation of plasmid DNA was by the alkali lysis method as described by Birnboim and Doly (1979) and Maniatis *et al.* (1982). Isolation of phage DNA was as described by Maniatis *et al.* (1982).

Construction of genomic libraries

*Eco*RI-digested DNA from the *a1-Mum2* strain was cloned in the λgtWES.λB vector (Leder *et al.*, 1977) as described by Wienand *et al.* (1981). For cloning of the *Mbo*I partial digest of the *a1-m(papu)* DNA the λEMBL4 vector (Frischauf *et al.*, 1983) was used as described by Schwarz-Sommer *et al.* (1984). The cloning of the wild-type *a1* allele from Line C and the *Mu* progenitor line was in the λNM1149 vector (Murray, 1983). The selection of recombinant clones by plague hybridization was by the method of Benton and Davis (1977). All subcloning of fragments from the recombinant clones was in the pUC9 plasmid (Vieira and Messing, 1982).

Recombinant plasmids

The recombinant plasmid pMJ9, used as source of the *Mu1* probe, was a gift from Michael Freeling and William C.Taylor, University of California, Berkeley, CA, and Jeffrey L.Bennetzen, International Plant Research Institute, San Carlos,

CA. The internal *Taq*I fragment of *Mu1* (Freeling, 1984) was subcloned from this plasmid into pUC9 and this fragment was used as probe in the cloning experiments. The plasmid pWx24 was the source of the *Spm-I8* probe (Schwarz-Sommer *et al.*, 1984). This plasmid contains a 2.5-kb *Sal*I fragment which contains most of the *Spm-I8* element. The plasmids p148-8 and p148-11, containing the *En1* 3.0-kb and 1.4-kb *Eco*RI fragments, respectively, from the autonomous *En1* element, were used as sources of the *En1* specific probes (Pereira *et al.*, 1985).

Southern hybridizations

Southern hybridizations (Southern, 1975; Wahl *et al.*, 1979) were carried out basically as described by Schwarz-Sommer *et al.* (1984) except that hybridizations were carried out at 68°C and filters were washed several times in 0.2 x SSPE, 0.1% SDS at 70°C.

Heteroduplex analysis

The procedure described by Davis *et al.* (1971) and Davis and Hyman (1971) was followed for heteroduplex analysis.

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References

Barker,R.F., Thompson,D.V., Talbot,D.R., Swanson,J. and Bennetzen,J.L. (1984) *Nucleic Acids Res.*, **12**, 5955-5967.
Bennetzen,J.L., Swanson,J., Taylor,W.C. and Freeling,M. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 4125-4128.
Benton,W.D. and Davis,R.W. (1977) *Science (Wash.)*, **196**, 180-182.
Birnboim,H.C. and Doly,J. (1979) *Nucleic Acids Res.*, **7**, 1513-1523.
Davis,R.W. and Hymann,R.W. (1971) *J. Mol. Biol.*, **62**, 287-303.
Davis,R.W., Simin,M. and Davidson,N. (1971) *Methods Enzymol.*, **21**, 413-428.
Döring,H.-P. and Starlinger,P. (1984) *Cell*, **39**, 253-259.
Fedoroff,N. (1983) in Shapiro,J. (ed.), *Mobile Genetic Elements*, Academic Press, NY, pp. 1-63.
Fedoroff,N., Wessler,S. and Shure,M. (1983) *Cell*, **35**, 235-242.
Fedoroff,N., Furtek,D.B. and Nelson,O.E. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 3825-3829.
Freeling,M. (1984) *Annu. Rev. Plant Physiol.*, **35**, 277-298.
Friedemann,P. and Peterson,P.A. (1982) *Mol. Gen. Genet.*, **187**, 19-29.
Frischauf,A.M., Lehrach,H., Poutska,A. and Murray,N. (1983) *J. Mol. Biol.*, **170**, 827-842.
Kirby,L.. and Styles,E.D. (1970) *Can. J. Genet.*, **12**, 934-940.
Leder,P., Tiemeier,D. and Enquist,L. (1977) *Science (Wash.)*, **196**, 175-177.
Maniatis,T., Fritsch,E.F. and Samborok,J. (1982) *Molecular Cloning, A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.
McClintock,B. (1951) *Carnegie Inst. Washington Yearbook*, **50**, 174-181.
McClintock,B. (1955) *Carnegie Inst. Washington Yearbook*, **54**, 245-255.
McClintock,B. (1957) *Carnegie Inst. Washington Yearbook*, **56**, 393-401.
McClintock,B. (1958) *Carnegie Inst. Washington Yearbook*, **57**, 415-429.
McClintock,B. (1961) *Carnegie Inst. Washington Yearbook*, **60**, 469-476.
McClintock,B. (1962) *Carnegie Inst. Washington Yearbook*, **61**, 448-461.
McClintock,B. (1968) *Carnegie Inst. Washington Yearbook*, **66**, 20-28.
Murray,N.E. (1983) in Hendrix,R.W., Roberts,R.W., Stahl,F.W. and Weisberg,R.A. (eds.), *Lambda II*, Cold Spring Harbor Laboratory Press, NY, pp. 395-432.
Nevers,P. Sherpherd,N.S. and Saedler,H. (1984) *Adv. Bot. Res.*, in press.
Nowick,E.M. and Peterson,P.A. (1980) *Maize Genet. Coop. Newsl.*, **54**, 3.
Nowick,E.M. and Peterson,P.A. (1981) *Mol. Gen. Genet.*, **183**, 440-448.
Pereira,A., Schwarz-Sommer,Zs., Gierl,A., Bertram,I., Peterson,P.A. and Saedler,H. (1985) *EMBO J.*, **4**, 17-23.
Peterson,P.A. (1961) *Genetics*, **46**, 759-771.
Peterson,P.A. (1970) *Theor. Appl. Genet.*, **40**, 367-377.
Peterson,P.A. (1981) *Cold Spring Harbor Symp. Quant. Biol.*, **45**, 447-455.
Reddy,L.V. and Peterson,P.A. (1984) *Mol. Gen. Genet.*, **194**, 124-137.
Robertson,D.S. (1978) *Mutat. Res.*, **51**, 21-28.
Schwarz-Sommer,Zs., Gierl,A., Klosgen,R.B., Wienand,U., Peterson,P.A. and Saedler,H. (1984) *EMBO J.*, **3**, 1021-1028.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-571.
Vieira,J. and Messing,J. (1982) *Gene*, **19**, 259-268.
Wahl,G.M., Stern,M. and Stark,G.R. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3683-3687.
Wienand,U., Langridge,P. and Fox,G. (1981) *Mol. Gen. Genet.*, **182**, 440-444.

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