The EMBO Journal vol.4 no.3 pp.591–597, 1985.

Plant transposable elements generate the DNA sequence diversity needed in evolution

Zsuzsanna Schwarz-Sommer, Alfons Gierl, Heinrich Cuypers, Peter A.Peterson[1] and Heinz Saedler

Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, FRG, and [1]Agronomy Department, Iowa State University, Ames, IA 50011, USA

Communicated by H.Saedler

Two germinal and 16 somatic reversion events induced by the Enhancer (En) transposable element system at the *wx-8*::Spm-I8 allele of *Zea mays* were cloned and studied by sequence analysis. Excision of the Spm-I8 receptor element from the *wx* gene results in various mutant DNA sequences. This leads to altered gene products, some of which are still capable of restoring the wild-type phenotype. Possible 'footprint' sequences that may have arisen by the excision of transposable elements were observed when intron sequences of the wild-type (wx^+) and the mutant (*wx-m8*) alleles of the *wx* gene were compared. The sequence divergence generated by visitation of a locus by plant transposable elements is discussed with respect to the molecular evolution of new gene functions.

Key words: Enhancer (En)/*wx-m8*/*Zea mays*/reversion/DNA sequence

Introduction

Transposable elements may be involved in developmental and evolutionary processes (McClintock, 1951, 1967, 1978; Nevers and Saedler, 1977; Fedoroff, 1984) because they can initiate a whole range of chromosomal rearrangements such as duplications, deletions, inversions, translocations, etc. (Döring and Starlinger, 1984; Nevers *et al.*, 1984) and they can influence the tissue specificity, developmental timing and level of gene expression. The present paper discusses a new aspect of transposable elements that may provide a clue to their biological significance.

All transposable elements studied so far are natural components of the genomes of living organisms and they can become activated by genomic stress (for references, see Fincham and Sastry, 1974; McClintock, 1984; Nevers *et al.*, 1984). Once activated they cause very rapid changes manifested by mutational instability of phenotypically selectable loci. Integration of a transposable element alters or abolishes expression of the gene in which insertion has occurred. Release from control by a transposable element can be detected as a reversion event (selectable as a stable wild-type phenotype) and is usually accompanied by excision of the element from the locus. In other cases excision of the element may lead to stable recessive mutations (Peterson, 1970; Nowick and Peterson, 1981). These may be due to the presence of a non-functional element at the locus that interferes with gene expression, or they may arise by excision events, altering the structure of the gene.

A number of cases have been examined in which excision of transposable elements occurred in germinal cells leading to progeny with a wild-type phenotype. Unexpectedly, these phenotypically wild-type revertants were found not to contain a wild-type DNA sequence of the locus. Instead excision of plant transposable elements seemed to have been imprecise, thus generating sequence diversity at the site of integration. This is true for the eight germinal revertants obtained from three different controlling element systems of *Antirrhinum majus* and *Zea mays* (see references below). However, in these instances either the precise location of the element with respect to the exon/intron structure of the gene was not known (Pohlmann *et al.*, 1984), or the element was located within control regions (Sachs *et al.*, 1983; Bonas *et al.*, 1984; Sommer *et al.*, in preparation) or within introns (Weck *et al.*, 1984). However, alterations of introns or control sequences are unlikely to affect the gene product, thus the intriguing question is whether alterations in sequences are produced and tolerated by excision of transposable elements from exons.

We demonstrated that in the (*waxy*) *wx-8*::Spm-I8 (*wx-m8*, McClintock, 1961) mutation of *Zea mays*, the receptor component, Inhibitor (=I, Peterson, 1953, 1960) of the Suppressor/Mutator (Spm, McClintock, 1954, =Enhancer= En, Peterson, 1965) controlling element system is inserted within an exon of the *wx* gene. In the absence of En or Spm the *wx* gene product is missing (Shure *et al.*, 1983), but in the presence of the autonomous element excision of the receptor component frequently occurs in somatic and germinal tissue and gene expression is restored (McClintock, 1961, Schwarz-Sommer *et al.*, 1984).

Here we report on the analysis of 18 DNA sequences generated by En-induced excision events at the *wx-m8* allele in germinal and somatic tissue. Sequence analysis of such events has shed light on the mechanism of transposition *via* excision and re-integration in plants (see accompanying paper by Saedler and Nevers). It has also offered some insights on how insertion and excision of transposable elements can lead to altered gene structures and thus to molecular evolution of a particular gene. By comparing intron sequences of two alleles of the *wx* gene we have been able to show that alterations in these sequences can readily be explained by frequent visitation of the introns by transposable elements. The possible significance of these 'footprints' with respect to evolution of DNA structures is discussed.

Results

Spm-I8 is located within an exon of the wx gene

The exon/intron structure of the wx^+ gene was determined by comparison of the DNA sequences of a cloned wild-type wx^+ cDNA with the genomic DNA sequences present in the wild-type wx^+ gene (Schwarz-Sommer *et al.*, 1984). In addition, the DNA sequence of the *wx-m8* allele was analysed. Figure 1 shows the exon/intron structure of the wx^+ gene around the target site of Spm-I8 within the *wx-m8* allele. A more detailed analysis dealing with the structure of the entire wx^+ gene is in preparation (Klösgen *et al.*, unpublished results).

The Spm-I8 insertion site within the *wx-m8* allele was found to be located within an exon of the *wx* gene (Figure 1). Our results revealed that the Ac-9 element of the *wx-m9* mutant (Fedoroff *et al.*, 1983) is also integrated in an exon, upstream of the loca-

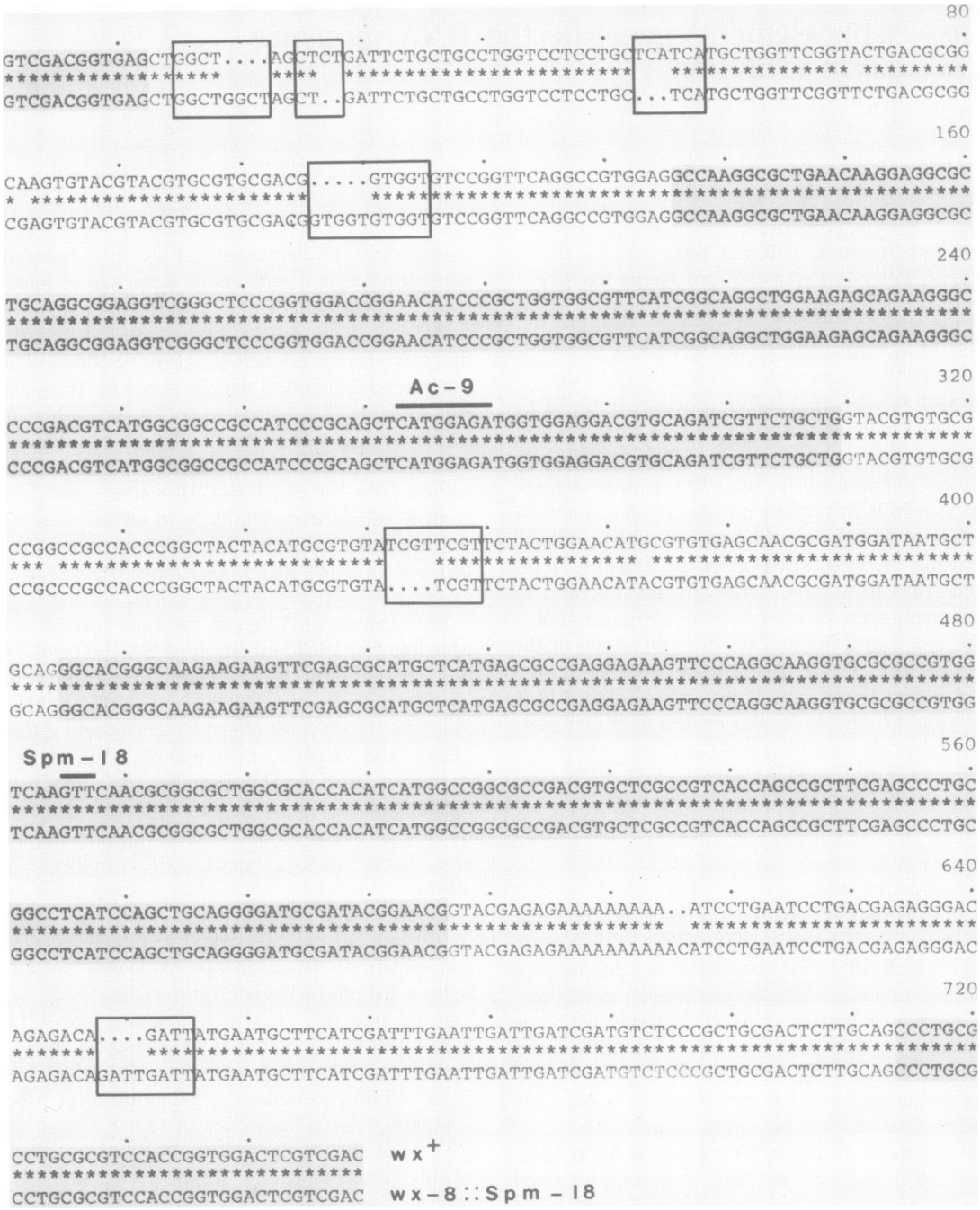

Fig. 1. Exon/intron structure of a portion of the *wx* gene within two alleles in *Z. mays*. The exons, indicated by shading, were identified by sequence analysis of a wild-type wx^+ cDNA obtained as described in Materials and methods. Discussion on the violation of consensus sequences of exon-intron boarders (see CT at position 13 – 14) will follow concerning the stucture of the entire wx^+ gene (Klösgen, unpublished results). The upper sequence was derived from the 0.75-kb *Sal* fragment of a wild-type (Line C) genomic wx^+ clone (Schwarz-Sommer *et al.*, 1984) and the lower sequence from the corresponding region of the *wx-m8* allele. Bases 0 and 749 are the first and last nucleotide of the sequenced 0.75-kb *Sal* fragment. The 5′ to 3′ direction of transcription is left to right. For sequence comparison of the two alleles the Spm-I8 insertion of the mutant allele was removed and the sequences were aligned to give maximal homology, as indicated by stars between homologous base pairs. Duplicated sequences present in the introns of one allele and missing in the other are boxed in. The targets for the Ac-9 (Fedoroff *et al.*, 1983) and Spm-I8 (Schwarz-Sommer *et al.*, 1984) insertions are indicated by bars covering the sequences duplicated in the corresponding mutant alleles.

tion of the Spm-I8 insertion. Comparison of the DNA sequences of the wild-type wx^+ and *wx-m8* alleles reveals that the sequences of the exons are conserved. The introns, however, show some deviations. There are either base substitutions or small sequence duplications in one allele and deletions in the other.

Sequence analysis of germinal revertants

By introducing an active En element into the genome of a plant carrying the *wx-m8* allele we succeeded in isolating several independent germinal revertants with Wx^+ phenotypes. The frequency of reversion was ~1%.

It is comparatively easy to prove that the revertants isolated did not arise due to contamination with wild-type pollen since the *wx-m8* allele contains a unique polymorphism which is not present in any other mutant or wild-type alleles studied so far (Schwarz-Sommer *et al.*, 1984 and unpublished results). This polymorphism is due to the presence of a 6-kb insertion (insert-1) near the 5′ end of the *wx* gene. The revertants were analysed

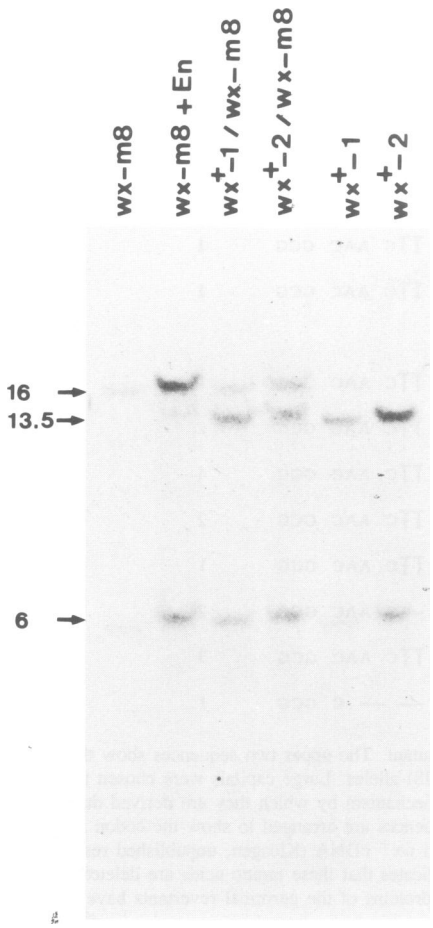

Fig. 2. Southern blot analysis of En-induced excision products generated at the *wx* locus in the *wx-m8* mutant. 5 μg of genomic DNA prepared from plants with the genotypes indicated above each lane was digested with *Eco*RI and loaded onto an 0.7% agarose gel. After electrophoresis the fragments were transferred onto nitrocellulose (Southern, 1975) and prehybridized and hybridized at 68°C as described earlier (Schwarz-Sommer *et al.*, 1984). The hybridization probe (a *Bam* 4.5-kb fragment of the wx^+ gene) was chosen to light up fragments containing insert-1 (6 kb, see Schawarz-Sommer *et al.*, 1984 and Results) and the 16-kb *Eco*RI fragment carrying Spm-I8 inserted into the *wx* gene. In the revertants the size of the latter fragment is 13.5 kb because Spm-I8 is excised.

by Southern hybridization procedures with a probe, covering insert-1 and the 5′ portion of the wx^+ gene. In *Eco*RI-digested genomic DNA the presence of insert-1 and the size of the revertant *wx* fragment can be demonstrated simultaneously. As shown in Figure 2, all genotypes carrying the *wx-m8* allele in homozygous or heterozygous form as well as the two revertants contain a 6-kb *Eco*RI fragment indicative of the presence of insert-1 (Schwarz-Sommer *et al.*, 1984). The homozygous *wx-m8*/*wx-m8* plant contains a 16-kb *Eco*RI fragment which is absent in the homozygous germinal revertants. By excision of the 2.2-kb Spm-I8 sequence in the revertants, a 13.5-kb *Eco*RI fragment appears instead.

The *wx*-specific 13.5-kb *Eco*RI fragment was cloned from two of the germinal revertants into the *Eco*RI restriction site of the λEMBL4 phage. The nucleotide sequence around the insertion site of Spm-I8 in these two revertants is compiled in Figure 3. In both cases excision of Spm-I8 did not result in restoration of the wild-type genomic DNA sequence but caused some rearrangements. The two revertant sequences are in-frame with the translation of the wx^+ mRNA, but both contain one additional triplet coding for either Leu or Ser (Figure 3).

Sequence analysis of somatic excision events occurring in leaf DNA

As shown in Figure 2 and already discussed in a previous report (Schwarz-Sommer *et al.*, 1984), frequent somatic excision events induced by En at the *wx-m8* allele lead to excision of the Spm-I8 insert and to almost precise restoration of the wild-type fragment size of the wx^+ gene in somatic leaf tissue. According to the intensity of the band appearing in Southern hybridization experiments, the frequency of excision events is estimated to be between 10% and 20% (Figure 2).

Using *Eco*RI-digested genomic RNA from leaves of plants with the genotype *wx-m8*+En, we cloned fragments of the 12 – 14 kb size range into the λ EMBL4 phage. Restriction analysis of nine recombinant phages showed that all had retained characteristic parts of insert-1. This supports our previous conclusion that insert-1 is not part of the En-I transposable element system (Schwarz-Sommer *et al.*, 1984).

Figure 3 shows the nucleotide sequences around the integration site of Spm-I8 in the nine isolates from somatic tissue. All excision products have an altered DNA sequence at this site compared with the wild-type wx^+ sequence. In three excision products the reading frame of the *wx* gene is altered. In six cases the mutations would lead to one additional amino acid within the protein. We found no precise excision events restoring the wild-type gene structure in this sample of somatic events in the leaf tissue.

Sequence analysis of somatic excision events within the endosperm at the cDNA level

The size of the wild-type wx^+ poly(A)$^+$ RNA is 2.4 kb (Shure *et al.*, 1983). This poly(A)$^+$ RNA is absent in the endosperm of *wx-m8* plants. Instead these plants contain a 3.2-kb chimeric message consisting of sequences from the 5′ portion of the *wx* gene and additional sequences hybridizing to Spm-I8 (Gierl *et al.*, 1985. After introducing En into the genome of *wx-m8* plants, a 2.4-kb *wx* mRNA of wild-type size appears in the endosperm at a level of ~10% of that obtained in wild-type wx^+ kernels (data not shown). This RNA was converted into cDNA and cloned into the *Eco*RI site of the λ NM1149 phage. Seven recombinant phages carrying *wx*-specific sequences were identified and sequenced.

The sequence data are included in Figure 3. Two out of the seven excision events precisely restored the wild-type wx^+ sequence. In three cases the first nucleotide (G) of the duplicated 3-bp sequence is retained and thus these cDNAs contain a frameshift mutation. In one case the complete duplicated target is left behind and in one other case the complete duplicated target plus three flanking nucleotides are deleted. These last events would not change the reading frame but the primary structure of the protein (Figure 3).

Discussion

Insertion and excision of plant transposable elements can alter the structure and function of genes

Insertion of plant transposable elements into genes can alter their structure and affect their expression. In cases where the function of the gene product could be analysed, it was found that the consequence of integration could range from total abolishment of gene expression to change in corresponding enzymatic properties or altered developmental control of gene expression (Dooner and Nelson, 1977; Schwartz and Echt, 1982). It follows that the physical presence of foreign DNA within an expressed gene alters its expression. The extent of this effect may depend

GENOTYPE	EVENTS	STRUCTURE OF PRODUCTS	ISOLATES	PROTEIN
wx^+		GTC AAG TT C AAC GCG		wildtype
wx-8::Spm-I8		GTC AAG TT——G TTC AAC GCG		*)
	germinal revertants			
wx^+-1		GTC AAG TTA - TTC AAC GCG	1	+ leu
wx^+-2		GTC AAG T- CG TTC AAC GCG	1	+ ser
	somatic excisions			
wx-8::Spm-I8 + En	a) in leaf DNA	GTC AAG T- ACG TTC AAC GCG	1	frameshift
		GTC AAG TT G TTC AAC GCG	2	+ leu
		GTC AAG TTA - TTC AAC GCG	4	+ leu
		GTC AAG T- G TTC AAC GCG	2	frameshift
	b) in endosperm RNA	GTC AAG TT G TTC AAC GCG	1	+ leu
		GTC AAG TT - --C AAC GCG	2	wildtype
		GTC AAG -- G TTC AAC GCG	3	frameshift
		GTC AA- -- - -- -- C GCG	1	△ lys, △ phe

Fig. 3. Compilation of sequences obtained by En-induced excision at the *wx* locus in the *wx-m8* mutant. The upper two sequences show the target of the Spm-I8 insertion (indicated by a bold line) within the wild-type (wx^+) and the mutant (*wx-8*::Spm-I8) alleles. Large capitals were chosen to symbolize the bases which are subject to alterations caused by excision events. These are arranged to show the mechanism by which they are derived during excision of the Spm-I8 element (see proposed model in the accompanying paper by Saedler and Nevers). The sequences are arranged to show the codon frame in the wx^+ mRNA which was derived from the single open reading frame present in the cloned and sequenced wx^+ cDNA (Klösgen, unpublished results). The symbol * indicates that a protein translated from the *wx-m8* template is not yet identified. The symbol △ indicates that these amino acids are deleted in a putative translation product. + stands for the addition of amino acids within a revertant protein. The wx^+ proteins of the germinal revertants have not yet been isolated and characterized.

on the position of the inserted element as well as on its particular structure (Peterson, 1976; Strommer *et al.*, 1982).

Excision of an integrated element may result in reversion to phenotypically wild-type gene expression. Classically one would have expected this to be due to restoration of a wild-type sequence at the locus in question. The observation that phenotypically wild-type revertants of transposable element induced mutations differ in the level of enzymatic activity of the gene products as well as in their kinetic properties or in their molecular weight (Dooner and Nelson, 1979; Echt and Schwartz, 1981; Tuschall and Hannah, 1982; Shure *et al.*, 1983) was unexpected.

Recently several phenotypically wild-type, germinal revertants obtained after excision of different transposable elements in different plants were examined at the DNA sequence level. In all of these revertants rearranged DNA sequences were found at the integration site of the excised elements (Sachs *et al.*, 1983; Bonas *et al.*, 1984; Pohlmann *et al.*, 1984; Weck *et al.*, 1984; Sommer *et al.*, 1985). Therefore it is reasonable to assume that the alterations in enzymatic activity mentioned above arose due to the effects of imprecise excisions. This already suggests that excision can generate alterations in sequences of a gene that are relevant for the function of the gene although the insertions analysed at the DNA sequence level occurred either in introns or in control regions.

As shown in Figure 1 the Ac-9 insertion in the *wx-m9* allele and the Spm-I8 insertion in *wx-m8* both occur within exons of the *wx* gene. Analysis of germinal revertants of these alleles reveals that excision of the elements does indeed alter the structure of the gene product. Excision of Ac-9 leaves a 6-bp duplication behind (Pohlmann *et al.*, 1984), which should lead to two additional amino acids in the revertant protein. Likewise, the Wx^+ proteins encoded by the two germinal revertants of the *wx-m8* allele must contain one additional amino acid each. Thus it can be concluded that insertion of plant transposable elements and subsequent excision can generate functionally active but structurally altered gene products. In view of the prevalence of imprecise excision in plants, it is remarkable that this phenomenon has not been observed in other systems such as bacteria or *Drosophila*.

With respect to the excision of bacterial transposons and insertion sequences, failure to observe imprecise excision in revertants may stem from a particular bias. It is commonly thought that a wild-type revertant of an insertion mutation located within the coding sequence of a bacterial gene can only arise by restoration of the wild-type DNA sequence. Hence, with the exception of one case in the IS4 system (Habermann *et al.*, 1979), no wild-type revertants of such insertion mutations have been sequenced. From the observation that integrated elements are flanked by small sequence duplications, it apparently seemed so obvious that these sequences simply recombine during excision that sequencing reversion products has been neglected. Failure to delve into the details of excision in bacteria can also be attributed to the fact that interest was focused on transposition, and until recently transposition and excision were believed to occur by separate pathways (see, for example, Berg and Berg, 1983). However, recent re-evaluation of the mechanism proposed in bacteria has shown that at least some of the elements also transpose *via* excision and integration (Bresler *et al.*, 1983; Morisato and Kleckner, 1984).

In *Drosophila* several germline revertants of mutations at the

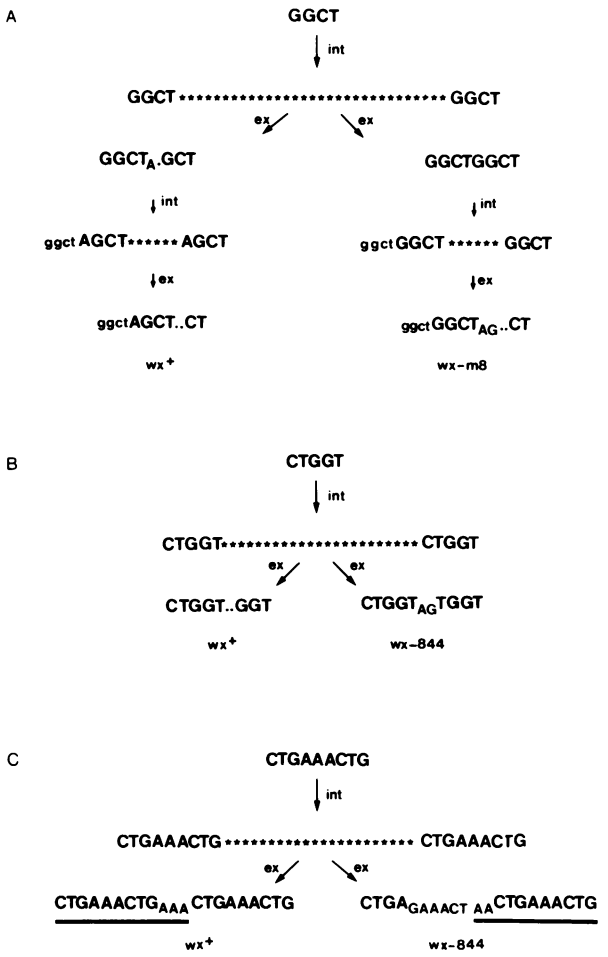

Fig. 4. Putative pedigree of duplicated sequences detected within introns of two alleles of the *wx* gene. A row of stars indicates the presence of a transposable element. Integration (int) of this element generates the target site duplication (bold capitals) in a putative ancestor. Excision(s) (ex) of the element leaves the altered targets behind which are now present in the analyzed lines. The mechanism of excision can be explained by the model presented in the accompanying paper by Saedler and Nevers. (**A**) Common pedigree of the duplications present at position 15–28 (Figure 1) in line C (wx^+) and in the line carrying the *wx-m8* allele. The small letters indicate the unchanged portion of the sequence which was left behind after the first visitation of this site. The bases depicted with small capitals could arise by template switch of DNA polymerase and the dots indicate exonuclease degradation of bases. (**B**) and (**C**) Pedigree of duplications obtained comparing intron regions within the 0.6-kb *Bam* fragment of the *wx* gene of line C (wx^+) and a line carrying the *wx-844* mutation (Pereira *et al.*, 1985). The altered duplication (**B**) present in the *wx-844* mutant allele can arise by exonuclease degradation and template switch occurring during DNA repair synthesis following excision at the right portion of the duplicated target. The mechanism leading to the sequences shown in **C** involves template slippage of DNA polymerase during repair synthesis after excision. This type of event is responsible for generation of altered sequences found in a revertant of the Tam3 insertion in *Antirrhinum majus* Sommer *et al.*, 1985). Since the target sequence contains the triplet CTG twice, a slippage by pairing of the CTGs of the new strand and the template is possible. After this event, the sequence shown by small capitals (see underlined portions in wx^+ and *wx-844*) would arise, provided exonuclease degradation of the template occurs in the meantime thus prohibiting complete repair synthesis. The other side of the duplicated target in wx^+ is repaired completely. The mechanism assumed to generate the left portion of the imprecise duplication found in the *wx-844* allele involves template slippage back to the base G after CTGA have been synthesised. The base G at the end is missing because of exonuclease degradation of the free template fringe.

white locus induced by P elements or foldback DNA were analysed (O'Hare and Rubin, 1983; Collins and Rubin, 1983). The four examples published represent precise excision events. This could indicate either that the mechanism of transposition and excision in *Drosophila* is different from the one proposed for plant elements (see accompanying paper by Saedler and Nevers) or one could argue that these insertions occur within regions of the gene which do not tolerate even in-frame alterations of the synthetised gene product. Hence selection for wild-type revertants necessarily leads to selection for precise excision events. Since evidence from other organisms is insufficient, it must remain open whether alterations of gene sequences *via* insertion and excision may be a general property of transposable elements.

Analysis of somatic excision events provides an explanation for the mechanism of transposition

As outlined in the accompanying paper, the altered structure left behind after excision of transposable elements in plants is a consequence of the mechanism of transposition. All revertant sequences compiled in Figure 3 are compatible with this model. Thus far no germinal reversions analysed contain an authentic wild-type gene sequence. This may be due to the small number of events sequenced. In contrast, among the 16 somatic excision events sequenced (Figure 3), two cases were found in which the wild-type sequence was restored. According to the model suggested by Saedler and Nevers, an event of this kind must be rare since it requires precise degradation of one of the flanking duplicated segments during excision, a process which is less probable than others.

Transposable elements generate some of the sequence divergence that may be valuable for evolution

As outlined above, plant transposable elements have been observed to generate sequence alterations at their sites of integration. This raises the following questions with respect to their role in the molecular evolution of genes: is the frequency of visitation by mobile elements in the plant genome high enough to be relevant for evolution? Is one of the steps, integration or excision, rate limiting?

The frequency of germinal and somatic excisions can be high (Peterson, 1970, 1978, 1981). In the case of the En-I transposable element system, as presented here, for example, it is 1% and 20%, respectively. Hence excision does not seem to be a rate-limiting step for the generation of mutations. To date no precise data are available concerning the frequency of insertions. If integration of only one particular plant element at a single locus is considered, estimates of integration frequency may be distorted by possible site specificity. This could be overcome by taking several different mobile elements in account. Furthermore, frequencies obtained for a given, selectable target may be misleading since only visible changes can be counted, which in turn represents a form of involuntary selection.

Thus to estimate the frequency of integration one should look for a region of the genome in which visitation could occur without serious consequences for gene expression. It follows that introns should constitute good targets for screening. According to the model of transposition of plant transposable elements presented in the accompanying paper (Saedler and Nevers), structures resulting from visitation by a transposable element are predictable and should consist of either perfect or imperfect sequence duplications.

We compared the intron regions within two alleles of the *wx* locus present in the maize line C (wx^+) and another line carrying the mutant *wx-m8* allele. As shown in Figure 1 the total length of the introns observed is ~300 bp. Within this comparatively short region of the genome we detected 11 differences between the two alleles. Six of these alterations are perfect duplications

of short sequences occurring either in the one or in the other allele, four are base substitutions and one is a 2-bp insertion. Thus the frequency of the mutation events caused by base substitutions equals that of small duplications. This is a minimum estimate since only the changes between the two alleles were scored and duplications already present in both lines, but presumably absent in a common progenitor, were neglected.

However, perfect duplications can also be the result of template splippage of DNA polymerase during the replication process. In contrast, imperfect duplications can only be explained by additional enzymatic processes like degradation by exonuclease or template switch of DNA polymerase. These events could only occur if DNA strands are open as is the case during excision of plant transposable elements (Saedler and Nevers, accompanying paper). Applying the rules of excision based on observed structures in germinal and somatic revertants, the two neighbouring duplications at the beginning of the first intron depicted in Figure 1 can be explained by two visitation events accompanied by imprecise excisions. As outlined in Figure 4A an initial visitation by a putative element may have occurred in a common ancestor of the two lines. Subsequently plant lines bearing slightly different excision products may have arisen. At this point the two lines may have been separated followed by visitation by a second element at the target produced by the first element. The sequences present in the two lines today are then the result of the second excision event.

This explanation is, of course, speculative. But it is supported by the results comparing another 200 bp of introns obtained by sequencing other regions of two alleles of the *wx* gene (Klösgen *et al.*, unpublished results). Here we found two more examples of imperfect duplications (Figure 4B and C) which are compatible with a mechanism involving insertion and excision of mobile elements.

Thus it is tempting to suggest that the duplicated sequences described above represent 'footprints' of mobile elements active in the genome of plants. There is no reason to doubt that similar visitations also take place within exon sequences with sufficiently high frequency. Obviously structural alterations generated by transposable elements rarely lead to improvement of the gene product in question and therefore are under selective pressure. Thus, duplication of the entire gene must precede mutation so that an intact copy of the gene can be conserved when the other is mutated. Interestingly transposable elements also seem to be capable of generating duplications (Courage-Tebbe *et al.*, 1983; Döring and Starlinger, 1984). A possible mechanism by which such duplications might arise has been described elsewhere (Nevers *et al.*, 1984).

Assuming that mobile elements play an important role in evolution by generating sequence divergence, the next step is to try to measure the frequency of such events. Comparison of the sequence of a gene from a plant raised from a 20 year old kernel with that of a plant propagated each year from a fresh kernel of the same line (thus separating the plants by 20 generations) should permit an estimation of the frequency with which sequence alterations occur during 20 years. Material of this kind is available in breeders stocks and we plan to examine it by comparing gene sequences as, for example, the wx^+ sequence.

Materials and methods

Genetic stocks

All genetic stocks were the same as previously described (Schwarz-Sommer *et al.*, 1984).

Germinal revertants were obtained by introducing En into *wx-m8*/*wx-m8* plants. Kernels showing a Wx^+ phenotype were selected and planted. The mature plants were then selfed. The phenotype of the progeny was determined by KI/I_2 stain. The frequency of revertant Wx^+ phenotypes was 1% in a population of ~600 kernels.

The nomenclature of the mutants and wild-type alleles used in this text corresponds in part to that used in bacterial genetics. Thus, the term Wx^+ indicates wild-type phenotype which may or may not have the wild-type genotype (which is wx^+ and wx^+-1 or wx^+-2 for its alleles). The symbol :: indicates the presence of insertion elements within the allele. For convenience this designation (like *wx-8*::Spm-I8) was used in the text or in Figures in an abbreviated form (e.g., *wx-m8*) which corresponds to the usual designation given by maize geneticists.

The methods applied in the studies reported in this paper (e.g., plasmid, phage and plant DNA preparation, Southern blot analysis and hybridizations, genomic cloning of plant DNA, sequence analysis) were performed as published earlier (Schwarz-Sommer *et al.*, 1984).

cDNA cloning

Endosperm RNA was prepared from kernels 18 days after pollination, as already reported (Schwarz-Sommer *et al.*, 1984). cDNA synthesis was performed following standard protocols (Land *et al.*, 1981; Maniatis *et al.*, 1982). After S1 treatment of the double-stranded cDNA, *Eco*RI linkers (BRL) were ligated to the blunt ends (Maniatis *et al.*, 1982). The ligation mix was digested with *Eco*RI (Boeheringer, Mannheim). The restriction digest containing 100 – 150 ng double-stranded cDNA was size fractionated on a 5 ml Sepharose 4B column (Maniatis *et al.*, 1982) and the first fractions containing 10 – 20% of the loaded cDNA amount, were pooled and ligated into the *Eco*RI site of the *lambda* NM1149 (Murray, 1983; Scherer *et al.*, 1981) and packaged as described previously (Wienand *et al.*, 1982).

Recombinant phages were selected by plating the packaged phages onto the hfl *E. coli* host POP 13b (obtained from V.Pirrotta, EMBL Heidelberg), which is a derivative of the strain POP 101 (Murray, 1983).

In a typical cloning experiment we obtained 2 – 5 x 10^5 p.f.u./10 – 20 ng of double-stranded, sized cDNA.

Acknowledgements

We thank Dr. Patricia Nevers for her help during preparation of this manuscript. We also thank Rita Berndtgen for her excellent technical assistance.

References

Berg,D.E. and Berg,C.M. (1983) *Biotechnology*, **1**, 417-435.
Bonas,U., Sommer,H. and Saedler,H. (1984) *EMBO J.*, **3**, 1015-1019.
Bresler,S.E., Tamm,S.E., Gonyshin,I.Yu. and Lanzov,V.A. (1983) *Mol. Gen. Genet.*, **190**, 139-142.
Collins,M. and Rubin,G.M. (1983) *Nature*, **303**, 259-290.
Courage-Tebbe,U., Döring,H.-P., Fedoroff,N. and Starlinger,P. (1983) *Cell*, **34**, 383-393.
Döring,H.P. and Starlinger,P. (1984) *Cell*, **39**, 253-259.
Dooner,H.K. and Nelson,O.E. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 563-5627.
Dooner,H.K. and Nelson,O.E. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 2369-2371.
Echt,C.S. and Schwartz,D. (1981) *Genetics*, **99**, 275-284.
Fedoroff,N. (1984) *Sci. Am.*, **250**, 64-74.
Fedoroff,N., Wessler,S. and Shure,M. (1983) *Cell*, **35**, 235-242.
Fincham,J.R.S. and Sastry,G.R.K. (1974) *Am. Rev. Genet.*, **8**, 15-50.
Gierl,A., Schwarz-Sommer,Zs. and Saedler,H. (1985) *EMBO J.*, **4**, 579-583.
Habermann,P., Klaer,R., Kühn,S. and Starlinger,P. (1979) *Mol. Gen. Genet.*, **175**, 369-373.
Land,H., Grez,M., Hauser,H., Lindenmaier,W. and Schütz,G. (1981) *Nucleic Acids Res.*, **9**, 2251-2266.
Maniatis,T., Fritsch,E.P. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.
McClintock,B. (1951) *Cold Spring Harbor Symp. Quant. Biol.*, **16**, 13-47.
McClintock,B. (1954) *Carnegie Inst. Washington Year Book*, **53**, 254-260.
McClintock,B. (1961) *Carnegie Inst. Washington Year Book*, **60**, 469-476.
McClintock,B. (1967) *Dev. Biol. Suppl.*, **1**, 84-112.
McClintock,B. (1978) *Stadler Symp.*, **10**, 25-47.
McClintock,B. (1984) *Science (Wash.)*, **226**, 792-801.
Morisato,D. and Kleckner,N. (1984) *Cell*, **39**, 181-190.
Murray,N.E. (1983) in Hendrix,R.W., Roberts,J.W., Stahl,F.W. and Weisberg,R.A. (eds.), *Lambda II*, Cold Spring Harbor Laboratory Press, NY, pp. 395-432.
Nevers,P. and Saedler,H. (1977) *Nature*, **268**, 109-115.
Nevers,P., Shepherd,N.S. and Saedler,H. (1984) *Adv. Bot. Res.*, in press.
Nowick,E.M. and Peterson,P.A. (1981) *Mol. Gen. Genet.*, **183**, 440-448.
O'Hare,K. and Rubin,G.M. (1983) *Cell*, **34**, 25-35.
Pereira,A., Schwarz-Sommer,Zs., Gierl,A., Bertram,I., Peterson,P.A. and Saedler,H. (1985) *EMBO J.*, **4**, 17-23.

Peterson,P.A. (1953) *Genetics*, **38**, 682-683.
Peterson,P.A. (1960) *Genetics*, **45**, 115-133.
Peterson,P.A. (1965) *Am. Naturalist*, **99**, 391-398.
Peterson,P.A. (1970) *Theor. Appl. Genet.*, **40**, 367-377.
Peterson,P.A. (1976) *Mol. Gen. Genet.*, **149**, 5-21.
Peterson,P.A. (1977) in Bukhari,A.I., Shapiro,J.A. and Adhya,S.L. (eds.), *DNA Insertion Elements, Plasmids and Episomes*, Cold Spring Harbor Laboratory Press, NY, pp. 429-435.
Peterson,P.A. (1978) in Walden,D.B. (ed.), *Maize Breeding and Genetics*, John Wiley and Sons, NY, pp. 601-631.
Peterson,P.A. (1981) *Cold Spring Harbor Symp. Quant. Biol.*, **45**, 447-455.
Pohlmann,R.F., Fedoroff,N.V. and Messing,J. (1984) *Cell*, **37**, 635-643.
Sachs,M.M., Peacock,W.J., Dennis,E.S. and Gerlach,W.L. (1983) *Mayidica*, **28**, 289-302.
Scherer,G., Telford,J., Baldari,C. and Pirrotta,V. (1981) *Dev. Biol.*, **86**, 438-447.
Schwartz,D. and Echt,C.S. (1982) *Mol. Gen. Genet.*, **187**, 410-413.
Schwarz-Sommer,Zs., Gierl,A., Klösgen,R.B., Wienand,U., Peterson,P.A. and Saedler,H. (1984) *EMBO J.*, **3**, 1021-1028.
Shure,M., Wessler,S. and Fedoroff,N. (1983) *Cell*, **35**, 225-233.
Sommer,H., Carpenter,R., Harrison,B.J. and Saedler,H. (1985) *Mol. Gen. Genet.*, in press.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.
Strommer,J.N., Hake,S., Bennetzen,J., Taylor,W.C. and Freeling,M. (1982) *Nature*, **300**, 542-544.
Tuschall,D.M. and Hannah,L.C. (1982) *Genetics*, **100**, 105-111.
Weck,E., Courage-Tebbe,U., Döring,H.-P., Fedoroff,N. and Starlinger,P. (1984) *EMBO J.*, **3**, 1713-1716.
Wienand,U., Sommer,H., Schwarz,Zs., Shepherd,N., Saedler,H., Kreuzaler, F., Ragg,H., Fautz,E., Hahlbrock,K., Harrison,B. and Peterson,P.A. (1982) *Mol. Gen. Genet.*, **187**, 195-201.

Received on 7 December 1984; revised on 4 January 1985