A Deletion Common to Two Independently Derived *waxy* Mutations of Maize

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ABSTRACT

A mutation at the maize *waxy* locus, *wx1240*, was isolated following treatment of pollen with EMS and self-pollinating ears on M1 plants. This allele was cloned and found to contain a 30-bp deletion within the gene and additional lesions upstream of the transcription start site. Using fine structure genetic mapping, we determined that the deletion is responsible for the mutant phenotype. In addition, the position of *wx1240* on the genetic map coincided with the previously determined positions of two other *waxy* mutations, the spontaneous *wx-C*, which is reference allele, and the putative ethyl methanesulfonate (EMS)-induced *wx-BL2*. Molecular analysis of these alleles revealed that both contain the same deletion as *wx1240*, and that the *wx-BL2* allele is similar to *wx-C* and possibly resulted from *wx-C* contamination. The deleted sequence responsible for these mutations is flanked by a short, 4-bp, direct repeat. Similar structures are favored sites for spontaneous deletions in other organisms. The data suggests that EMS is capable of inducing structural alterations in plant genes in addition to the point mutations normally ascribed to EMS-induced mutations.

THE *waxy* (*wx*) locus of *Zea mays* encodes a starch biosynthetic enzyme responsible for the synthesis of amylose in the endosperm, pollen and embryo sac. Mutant *waxy* alleles are viable and easily distinguished. For this reason, over 50 *wx* mutant alleles have been identified since the turn of the century. The *wx* mutations, which all map to the short arm of chromosome *9*, represent one of the best collections of mutant alleles among higher plants. Molecular characterization of many of these alleles has provided the first comprehensive study of the lesions that underlie plant mutations. For example, the transposable elements *Activator (Ac)*, *Dissociation* (*Ds*) and *Suppressor-mutator* (*Spm*) were cloned from unstable *wx* alleles (FEDOROFF, WESSLER and SHURE 1983; PEREIRA *et al.* 1985). In addition, analysis of stable *wx* mutants demonstrated that spontaneous mutations in maize usually involved the insertion or deletion of DNA (WESSLER and VARAGONA 1985). Unlike these spontaneous mutations, none of the induced alleles have been characterized at the molecular level. In order to have a more complete picture of mutation in higher plants, we undertook the isolation and characterization of ethyl methanesulfonate (EMS)-induced *wx* alleles.

In *Escherichia coli* almost all EMS-induced mutations are point mutations that are produced when O^6-alkylguanine mispairs with thymine; all 184 mutations of the *LacI* gene were single base pair substitutions, 183 were a G:C to A:T transition (BURNS, ALLEN and GLICKMAN 1986). Similar results have been reported in eukaryotes. For example, in a study using EMS-treated human cell lines, all 54 mutations characterized were single base pair substitutions; 53 events changed a G:C base pair (LEBKOWSKI, MILLER and CALOS 1986). Similarly, EMS-induced mutations of the *Drosophila melanogaster rosy* locus (COTE *et al.* 1986) and the maize *adh1* locus (HAKE, TAYLOR and FREELING 1984) have been found to result from apparent point mutations. Although point mutations seem to be the rule, a few exceptions have been noted; in a few instances insertions or deletions were found (LACY, EISENBERG and OSGOOD 1986; MOGAMI *et al.* 1986; COTE *et al.* 1986)

In this study, the *wx* alleles *wx1240* and *wx1050* were isolated following EMS mutagenesis. Southern blot analysis of these alleles revealed multiple alterations in the gene structure; these changes could not be explained by a single lesion (M. VARAGONA and S. R. WESSLER, unpublished data). This finding was in marked contrast to the results from other organisms mentioned above and prompted further study of these mutations.

MATERIALS AND METHODS

Maize strains: The *wx* alleles *wx1240* and *wx1050* were induced as described below. Additional *wx* alleles were obtained from O. E. NELSON and included *wx-BL2*, an EMS-induced allele isolated by BRIGGS, and the spontaneous

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mutations *wx-C*, *wx-B1* *wx-M*, *wx-Stonor*, *wx-Cl*, *wx-R*, *wx-I* and *wx-H21* (NELSON 1968, 1976). Nonmutant alleles were from the inbred lines HY, W23, GA 209 and a full colored derivative of W22 called Line C by P. PETERSON (these alleles are identified here by the inbred line, for example, *Wx-W23*, *Wx-Hy*). *Wx-HY* is the cloned allele (SHURE, WESSLER and FEDOROFF 1983) and *Wx-LineC* is the sequenced allele (KLOSGEN *et al.* 1986).

Isolation of *wx1240*: The male parent had the genotype *A/A Wx/Wx C/c R/r*, and the female parent *A/A Wx/Wx c/c* and *r/r*. Mature pollen was collected and mixed with 1% EMS solution in paraffin oil for 45 min. The treated pollen was then brushed onto fresh silks (NEUFFER and COE 1978). Two new *wx* mutants, designated *wx1050* and *wx1240*, were identified segregating on separate ears of these M1 plants and maintained by self-pollination. Progenitor alleles were also maintained by self-pollination; the progenitor stock contained two nonmutant *Wx* alleles (as distinguished by restriction fragment length polymorphisms) that were designated *Wx1240p* and *Wx1050p*. DNA for this study was isolated from plants four or five generations removed from the induction of the mutations.

Molecular analysis of *wx* alleles: Isolation of genomic DNA, Southern blot analysis and screening of genomic libraries were as described previously (OKAGAKI and WESSLER 1988). The pBamC and pSst fragments (see Figure 2) used to probe Southern blots were subcloned from an 11-kb *Eco*R1 fragment which contains the entire *Wx* gene and about 5 kb of 5′ flanking sequences (SHURE, WESSLER and FEDOROFF 1983).

Southern blot analysis identified a 9.4-kb *Hin*dIII fragment suitable for cloning the entire *wx1240* allele. *Hin*dIII digested genomic DNA was cloned in the lambda vector 2001 (Stratagene) according to the supplier's directions, packaged with the Gigapack kit (Stratagene) and plated on LE 392 cells. Two positive plaques out of 200,000 primary transfectants were identified. The 9.4-kb *Hin*dIII insert was subcloned into pUC119 (VIEIRA and MESSING 1987) for further study.

The 2.1-kb *Sal*I fragment containing the *wx-BL2* lesion was cloned by digesting 50 μg of genomic DNA with *Sal*I, gel purifying fragments between 2 and 3 kb and cloning this mixture into the vector lambda Zap that had been digested with *Xho*I and phosphorylated (Stratagene). Of a primary library of 80,000 plaques screened with *wx* probe II (WESSLER and VARAGONA 1985) 15 positives were identified. A 600-bp *Sst*I fragment, containing the mutated region was subcloned into pUC118 (VIEIRA and MESSING 1987) for DNA sequencing.

The DNA containing the *wx-C* lesion was isolated following polymerase chain reaction (PCR) amplification using conditions described previously (KIM and SMITHIES 1988). Samples of 150 ng of genomic DNA and 700 ng of primers were cycled 45 times for 30 sec at 94° then for 6 min at 72° followed by a final incubation of 5 min at 72°. Primers for the PCR amplification, 5′-CGCGTGTTCGTTGACCACCC-3′ and 3′-AGGCGCTGAACAAGGAGGCG-5′, were derived from the published *Wx* sequence (KLOSGEN *et al.* 1986). Amplified DNA was digested with *Sst*I and subcloned into pUC119 for further analysis.

Selected regions of the *wx* alleles were sequenced using the dideoxy procedure (SANGER, NICKLEN and COULSEN 1977). Single stranded templates for sequencing were produced from inserts cloned into pUC118 or pUC119 (VIEIRA and MESSING 1987).

Fine structure genetic mapping: The lesion responsible for the mutant phenotype in *wx1240* was located by fine structure mapping (NELSON 1968). Seven *wx* alleles that together span the genetic map of the locus were selected, and plants containing these alleles were crossed with *wx1240* plants. Mature tassels from the F_1 plants were collected for pollen analysis and stored at −20° in 70% ethanol. Pollen was isolated from unopened spikelets, stained with potassium iodide, and examined under a microscope (NELSON 1968). Non-mutant pollen grains are black and *wx* grains reddish brown. This technique can reliably measure recombination down to a level of 1:100,000; below this frequency, black staining pollen is occasionally detected between alleles with overlapping deletions (NELSON 1968; RALSTON, ENGLISH and DOONER 1987). This is most likely due to staining artifacts or contamination by foreign pollen.

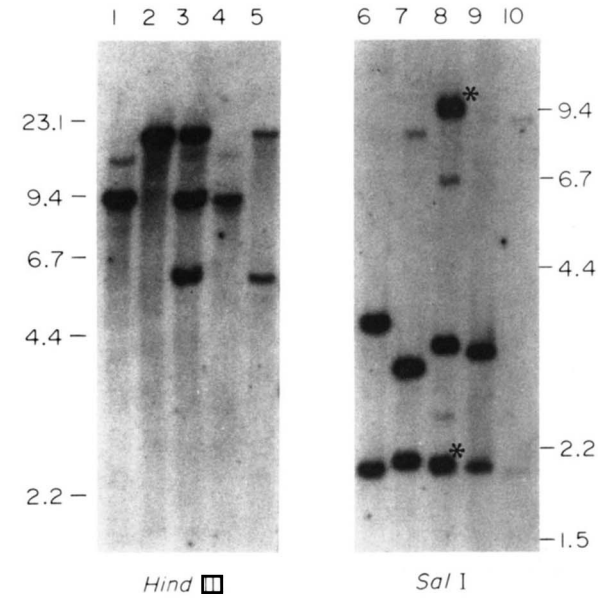

FIGURE 1.—Southern blot characterization of *wx* alleles associated with EMS mutagenesis and their progenitors. DNA samples were digested with either *Hin*dIII (lanes 1–5) or *Sal*I (lanes 6–10) and Southern transfers were hybridized with the pBamC probe (Figure 2). Lanes 1 and 6, *wx1240*; lanes 2 and 7, *wx1050*; lanes 3 and 8, *Wx1240p/Wx1050p*; lanes 4 and 9, *Wx1240p*; lanes 5 and 10, *Wx1050p*. The faint *Sal*I bands present in *Wx1050p*, lane 10, are starred in the heterozygous progenitor, lane 8. Slight differences in the migration of fragments from the *Sal*I digested progenitors, lanes 8–10, are gel artifacts; these fragments comigrate on other gels and the individual progenitor alleles were recovered by selfing the heterozygous progenitor.

RESULTS

Recovery of *wx* mutants following EMS mutagenesis: Two *wx* alleles, designated *wx1240* and *wx1050*, were recovered from a population of 3172 treated gametes. It is unlikely that these alleles were spontaneous mutations because of the high frequency of occurrence. Spontaneous mutation frequencies in maize are 2 to 3 orders of magnitude lower than the average mutation frequency of 1×10^{-3} observed for *wx*, *sul*, *clfI* and *nec3* mutations following EMS mutagenesis of pollen (NEUFFER and COE 1978). The frequency of spontaneous *wx* mutation has been estimated to be 1.1×10^{-5} (ROBERTSON 1985) and lower (STADLER 1932).

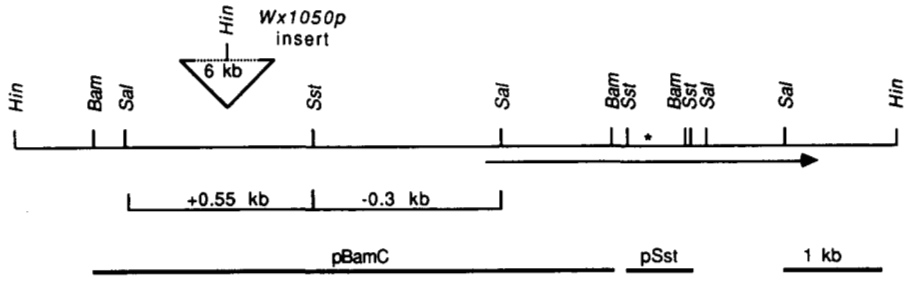

FIGURE 2.—Physical map of *Wx1240p*, *wx1240* and *Wx1050p*. The arrow delimits the approximate limits of the transcription unit and an asterisk indicates the position of the deletion in *wx1240*. Two upstream polymorphisms between *wx1240* and *Wx1240p* are shown immediately below the restriction map. The 5′ *Sal*I-*Sst*I fragment in *wx1240* is approximately 0.55 kb larger than the corresponding fragment in *Wx1240p*, and the 3′ *Sal*I-*Sal*I fragment is approximately 0.3 kb smaller in *wx1240*. These alterations are upstream of the normal transcription start site (data not shown). The major difference between the progenitors *Wx1050p* and *Wx1240p* is the 6-kb insertion in a *Sal*I-*Sst*I fragment indicated by the triangle above the restriction map. Southern blot analysis of *Wx1050p* and *wx1050* suggests that this insertion has excised. The two fragments used for making radiolabeled probes, pBamC and pSst, are shown at the bottom of the figure; pBamC is the 5′ *Bam*HI fragment subcloned from *Wx-Hy* (SHURE, WESSLER and FEDOROFF 1983) and pSstI was subcloned from *wx1240*. Restriction enzymes are *Bam*, *Bam*HI; *Hin*, *Hin*dIII; *Sal*, *Sal*I; *Sst*, *Sst*I.

Southern blot analysis (Figure 1) also rules out the possibility that these alleles are contaminants since they contain rearrangements of *wx* sequences not seen among a large collection of previously characterized *wx* mutations (WESSLER and VARAGONA 1985). The pBamC probe (Figure 2) detects a *Hin*dIII fragment of approximately 9.4 kb in the mutant *wx1240* (Figure 1, lane 1) and its progenitor *wx1240p* (lane 4). However, the two *Sal*I fragments detected by pBamC (Figure 2) differ in size when *wx1240* (lane 6) and *Wx1240p* (lane 9) are compared. Similarly, *wx1050* (lanes 2 and 7) differs from its progenitor *Wx1050p* (lanes 5 and 10) when both *Hin*dIII and *Sal*I digests are compared. The differences between the progenitors *Wx1240p* and *Wx1050p* is caused by an insertion of approximately 6 kb upstream of the start of *Wx* transcription (Figure 2) (WESSLER and VARAGONA 1985). This insert contains a *Hin*dIII site but not a *Sal*I site. The molecular lesions responsible for the EMS-induced mutations were too complex to discern by Southern blot analysis, however, they clearly demonstrated that the physical structures of *wx1240* and *wx1050* were different from previously characterized *wx* alleles (WESSLER and VARAGONA 1985).

Physical mapping of *wx1240*: We focused our attention on *wx1240*, and as an initial step cloned the allele on a 9.4-kb *Hin*dIII fragment (see MATERIALS AND METHODS). After subcloning into pUC119 (VIEIRA and MESSING 1987) a restriction map of the 9.4-kb *Hin*dIII fragment was generated. A comparison of the restriction maps of the cloned *Hin*dIII fragment and its progenitor, as derived from genomic Southern blots, indicated that the *wx1240* allele differed from its progenitor in two ways: (i) there was a small deletion in a 0.6-kb *Sst*I fragment near the middle of the gene (Figure 2) and (ii) there were several alterations at the 5′ end of the gene. These upstream changes could be explained by a duplication, a deletion, and the loss of a *Sst*I site. However, we have no data supporting any particular sequence of event(s) and have simply indicated the differences in restriction fragment sizes detected by pBamC (Figure 2).

Genetic mapping of *wx1240*: To determine which of the lesions was responsible for the mutant phenotype, fine structure genetic mapping was undertaken. Mutant *wx1240* plants were crossed with seven different *wx* alleles: *wx-B1*, *wx-M*, *wx-Stonor*, *wx-C1*, *wx-R*, *wx-I* and *wx-H21*. The location of these alleles, which span almost the entire genetic map of the *waxy* locus (NELSON 1968, 1976), are shown in Figure 3A. When the pollen of the various heteroalleles was stained with I_2/KI, revertant pollen was found in the crosses with *wx-B1*, *wx-M*, *wx-C1*, *wx-R*, *wx-I* and *wx-H21* but not with *wx-Stonor* (Table 1). Thus, the lesion responsible for the *wx1240* mutation must be coincident with or near the *wx-Stonor* lesion.

With the physical positions of several of the *wx* alleles used for mapping known (*wx-B1*, *wx-Stonor*, *wx-M* and *wx-I*) (WESSLER and VARAGONA 1985; WESSLER *et al.* 1990; M. VARAGONA, M. PURUGGANAN and S. R. WESSLER, unpublished data), the genetic map can be correlated with the physical map and the approximate position of the lesion responsible for the *wx1240* mutant phenotype can be determined. The *wx-Stonor* mutation is a large insertion that is 100 bp upstream of the 0.6-kb *Sst*I fragment harboring the deletion (Figure 3B; M. VARAGONA, M. PURUGGANAN and S. R. WESSLER, unpublished data). The fact that *wx1240* can recombine with *wx-B1*, a deletion extending from −655 to +299 (Table 1; WESSLER *et al.* 1990), suggests that the *wx1240* RFLPs in this region do not contribute to the mutant phenotype.

To precisely define the mutant lesion, a 1-kb region extending from 315 bp upstream of the *Sst*I site (including the *wx-Stonor* insertion site, Figure 3B) to

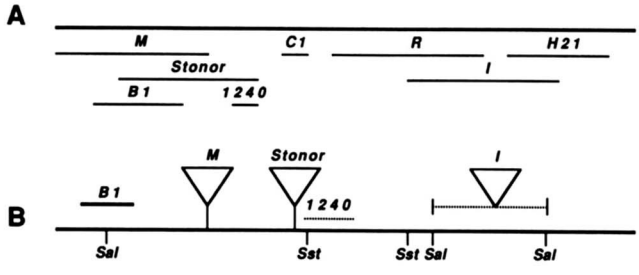

FIGURE 3.—Correlation of genetic and physical maps of *wx1240*. (A) The genetic fine structure map is based on the work of NELSON (1976). *wx1240* was mapped against seven alleles covering almost the entire *waxy* locus; this work placed *wx1240* adjacent or downstream of *wx-Stoner*. (B) Restriction map showing the positions of a deletion and three insertion mutations with the predicted position of *wx1240*. The position of the deletion, *wx-B1* (WESSLER *et al.* 1990) is represented by the heavy line. The positions of *wx-M* and *wx-Stonor* were determined by cloning the alleles and sequencing the insertion sites (M. J. VARAGONA, unpublished data); the approximate position of *wx-I* was determined previously (WESSLER and VARAGONA 1985). The dotted line represents the approximate position of the *wx1240* mutation.

TABLE 1

The frequency of *Wx* pollen grains in various *wx(a)/wx(b)* heterozygotes

	Cross	*Wx*/total pollen grains
wx1240/wx-B1	1354B	17/84,483
	87.40-#1	6/65,053
	87.40-#2	6/54,506
	Total	29/204,042
wx1240/wx-M	1353A	10/90,733
	87.39A	9/77,480
	Total	19/168,213
wx1240/wx-Stonor	1352A	0/80,340
	87.38-1	0/102,667
	87.38-2	1/88,632
	Total	1/271,639
wx1240/wx-C1	1350B	9/53,500
	87.36	21/116,667
	Total	30/170,167
wx1240/wx-R	1351A-#1	13/53,660
	1351A-#2	10/72,000
	Total	23/125,660
wx1240/wx-I	1349B	22/104,792
	87.35-6	31/89,960
	Total	53/194,752
wx1240/wx-H21	1348A	13/51,958
	1348B	50/100,995
	Total	63/152,953
wx1240/wx-C	GH35007-1	1/68,550
	GH35007-2	0/84,280
	GH35007-3	0/63,500
	GH35008	0/68,150
	Total	1/284,480

750 bp downstream was sequenced. Comparison of this sequence with the published *Wx* sequence (KLOSGEN *et al.* 1986) identified four single base substitutions (data not shown), a 4-bp deletion in intron 6

FIGURE 4.—Deletion in *wx1240* and *wx-C*. (A) The mutant lesion in *wx1240* and *wx-C*. The top line is the sequence from the nonmutant *Wx-LineC* allele (KLOSGEN *et al.* 1986); underneath is the sequence from *wx1240* and *wx-C*. The deletion removes one copy of a 4-bp direct repeat that flanks the deletion. (B) A deletion polymorphism. In *wx1240* and *wx-C* there are three copies of the 4-bp tandem repeat, TCCA; in *Wx-LineC* there are two copies of this repeat. Upper case letters represent exon sequences and lower case letters represent intron sequences.

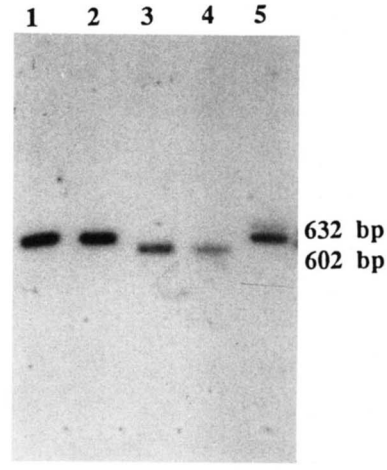

FIGURE 5.—Southern blot showing the deletion in *wx1240*. Genomic and cloned DNAs were digested with *Sst*I and probed with pSst (Figure 2). The cloned *wx1240* fragment (lane 4) comigrates with the corresponding genomic DNA fragment (lane 3), confirming that the deletion is not a cloning artifact. The faint band in the *Wx-Hy* lane may be due to a third *Sst* site 30 bp downstream of the 0.6-kb *Sst*I fragment. Lane 1, *Wx-Hy*; lane 2, *Wx1240p*; lane 3, *wx1240*; lane 4, cloned *wx1240*; lane 5, cloned *Wx-Hy*.

(Figure 4B) and a 30-bp deletion removing the last 4 bp of exon 7 (Figure 4A). This latter deletion is presumably responsible for the mutant phenotype.

The same deletion in *wx1240* and *wx-C*: The sequence deleted in *wx1240* is bounded by a 4-bp direct repeat (Figure 4A). This feature is characteristic of spontaneous deletions in bacteria and other organisms (FARABAUGH *et al.* 1978; EFSTRATIADIS *et al.* 1980). This suggests that the *wx1240* deletion could be a sequence prone to delete. Southern blot analysis comparing genomic DNAs from *wx1240* and its progenitor to the cloned alleles eliminated the possibility of a cloning artifact (Figure 5), and encouraged us to look for other *wx* alleles with this deletion.

The genetic fine structure map suggested other alleles that may have the same deletion as *wx1240*. *wx-BL2*, an EMS-induced allele, and *wx-C*, a spontaneous

mutant, are located in the region where *wx1240* is located (NELSON 1976). Examination of pollen from *wx1240/wx-C* heterozygotes revealed no recombination between these two alleles (Table 1). In addition, NELSON (1976) in an earlier study found no recombination between *wx-BL2* and *wx-C*. Previous Southern blot characterization of *wx-BL2* and *wx-C* determined that they were indistinguishable from the wild-type *Wx-Hy* allele however, a 30-bp deletion was below the limits of resolution in the study (WESSLER and VARAGONA 1985). To determine if these alleles contained a similar deletion, a 2.1-kb *Sal*I fragment containing this region from *wx-BL2* was cloned into the lambda ZAP vector (Stratagene). Similarly, appropriate upstream and downstream primers were used in conjunction with PCR to amplify this region in *wx-C* containing genomic DNA (see MATERIALS AND METHODS). The DNA sequences spanning the deletion breakpoints were determined and revealed that all three *wx* alleles contain precisely the same deletion (Figure 4A).

We are confident that *wx1240* and *wx-C* represent independent mutational events for the following reasons. First, *wx1240* was uncovered by self-pollinating EMS-treated material, not by crossing to a *wx* mutant. Second, 5′-flanking polymorphisms distinguish *wx1240* from *wx-C* (WESSLER and VARAGONA 1985). Polymorphisms are common in the 5′ *Sal*I fragment. This fragment is 3.2 kb in *Wx-Hy* and approximately 4 kb in *wx1240* (Figure 1, lane 6). In contrast, there are no 5′ RFLP differences between *wx-BL2* and *wx-C* (WESSLER and VARAGONA 1985), suggesting that these could be the same allele. The *wx-BL2* allele was isolated by BRIGGS following EMS-mutagenesis (NELSON 1968). However, unlike the selfing of M1 plants in this study, many of the alleles induced by BRIGGS were trapped by crossing the mutagenized plants with a *wx* tester (BRIGGS, AMANO and SMITH 1965). Since *wx-C* is the *wx* reference allele, it is likely that it was the tester used and what is now called *wx-BL2* is actually *wx-C*.

DISCUSSION

The ability to genetically map *wx* mutants with relative ease has facilitated the experiments described in this study. Comparison of the *wx1240* allele with its *Wx* progenitor revealed that it had multiple lesions. By genetically mapping *wx1240* relative to several previously characterized *wx* alleles, we determined that a 30-bp deletion was responsible for the mutant phenotype. Furthermore, the genetic proximity of *wx1240* and the previously mapped *wx* alleles *wx-BL2* and *wx-C*, led to the cloning of these alleles and the finding that they contained the same deletion and were probably identical alleles.

Although *wx1240* is a complex mutation with a deletion and additional alterations upstream of the transcription start, it is a formal possibility that all of the lesions were not induced by EMS. About five generations intervened between the time of EMS treatment and the time *wx1240* DNA was first characterized. It is conceivable, though unlikely, that a second, spontaneous mutation occurred during this time and contributed to the complexity of *wx1240*. Even if this were the case, the finding that the deletion is the lesion responsible for the mutant phenotype strongly suggests that it was EMS-induced because it was present in the generation immediately after EMS treatment.

Slipped mispairing and deletion formation in maize: The finding of a small deletion in the spontaneous allele *wx-C* is consistent with results from nonplant systems. The sequence deleted in *wx1240* and *wx-C* is flanked by a 4-bp direct repeat (Figure 4A). Short direct repeats have been associated with spontaneous deletions in several organisms from *E. coli* (FARABOUGH *et al.* 1978) to humans (EFSTRATIADIS *et al.* 1980). It has been postulated that deletion formation initiates when short direct repeats align out of register during DNA replication. This process is called slipped mispairing (STREISINGER *et al.* 1966). When direct repeats are in tandem arrays, repeat units may be added or deleted; the repeat of the intron sequence TCCA, which is found two times in *Wx-LineC* and three times in the other alleles (Figure 4B), may be an example of slipped mispairing. When repeats are separated, rather than in tandem arrays, the intervening DNA and one of the repeats may be deleted. In these cases, dyad symmetry within the intervening DNA can contribute to deletion formation by bringing repeats together and facilitating the mispairing (GLICKMAN and RIPLEY 1984). Structures of this type have been shown to be preferred sites for deletion formation (ALBERTINI *et al.* 1982). However, for the deletion reported here, the possible stem structures are unlikely to be stable.

Although the occurrence of the same deletion in two, and possibly three, independent alleles suggests that this might be a favored site for deletion formation, the presence of this deletion in both spontaneous and EMS-induced mutations is surprising. Normally EMS creates point mutations by a two-step process (LOVELESS 1969). First, alkylation of the O^6 position of guanine forms a premutagenic lesion; most of these alkylated bases are subsequently repaired. Second, alkylated guanines that persist pair with thymine residues during DNA replication and result in base substitutions. The sequences of EMS-induced mutations is fully consistent with this model.

How can this process result in the 30-bp deletion? The three guanine residues next to the upstream

member of the direct repeat (Figure 4A) may provide a target for EMS-mediated alkylation. Excision repair would then be postulated to remove the alkylated base and create a stretch of single stranded DNA. Excision repair has been shown to be involved in the repair of EMS-modified DNA in *E. coli* (TODD, BROUWER and GLICKMAN 1981). Direct repeats within the gap of single stranded DNA produced during excision repair could undergo slipped mispairing in the same way as slipped mispairing occurs during DNA synthesis. We have proposed that the misalignment occurs during repair rather than during DNA synthesis because the EMS-treated maize pollen does not undergo replication until after fertilization (WEATHERWAX 1917).

Although most EMS-induced mutations characterized to date are point mutations, EMS-induced deletions and insertions have been reported in nonplant systems. For example, of fourteen presumptive mutations in the Drosophila myosin heavy chain gene, three were insertions and one was a deletion (MOGAMI *et al.* 1986). In addition, of 29 EMS-induced mutations of the Drosophila *RPII215* locus, one was a deletion of about 0.6 kb (LACY, EISENBERG and OSGOOD 1986). Finally, EMS is not the only alkylating agent capable of inducing these structural changes; of seven *N*-ethyl-*N*-nitrosourea (ENU)-induced mutations of the Drosophila *rosy* locus, five were base substitutions, one a deletion of 1 bp and one a deletion of 56 bp (LEE *et al.* 1987). There were no direct repeats associated with the deleted sequence. Two additional deletions were also detected from a group of 20 ENU-induced *RPII215* mutations (LACY, EISENBERG and OSGOOD 1986).

Most studies of EMS-induced mutations in eukaryotes utilize Southern blots to discern whether a mutation is a base change or a more obvious lesion (LACY, EISENBERG and OSGOOD 1986; COTE *et al.* 1986; HAKE, TAYLOR and FREELING 1984). Our finding that a 30-bp lesion, virtually undetectable on genomic Southern blots, is responsible for both a spontaneous mutation and a mutation associated with EMS mutagenesis, suggests that prior studies based on Southern blot analysis may be underestimating the role of small deletion in induced and spontaneous mutations in eukaryotes.

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