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Tissue-Specific Accumulation of MURB, a Protein Encoded by *MuDR*, the Autonomous Regulator of the *Mutator* Transposable Element Family

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The *Mutator* (*Mu*) system of transposable elements is highly mutagenic and can maintain high levels of activity through multiple generations due to frequent transpositions of both its autonomous and nonautonomous components. This family also shows pronounced developmental regulation. Most notable is the very low frequency of germinal reversions, despite the high levels of somatic transpositions and excisions, and the high frequency of germinally transmitted duplication events. Here, we report the production of antibodies raised against MURB, one of two proteins encoded by *MuDR*, the autonomous regulator of the *Mu* family. Immunolocalizations performed using anti-MURB antibodies reveal that this protein is present in specific tissues during male inflorescence development. Throughout much of development, MURB is detected at the highest levels in cell lineages that may find themselves in the germ line, but no MURB is detected in microspore mother cells. These cells are the direct precursors to pollen. Based on these observations as well as previous data, we discuss the relationship between the expression of MURB and developmental regulation of *Mu* activity.

INTRODUCTION

The complex relationship between transposable elements and their hosts involves a dynamic equilibrium between the ability of transposons to increase their copy number rapidly and the opposing negative selection against copy number amplification (reviewed in Charlesworth and Langley, 1989). The effects of transposon activity can vary significantly, depending on whether that activity results in heritable changes. Transposon amplification in somatic lineages can result in damaging effects on the host without increasing the heritable copy number of the transposon. Transposon activity specifically in the germ line has the obvious advantage of resulting in a heritable copy number increase. Therefore, transposons that can distinguish between these two lineages will best maximize their heritable copy number at minimum cost to their hosts.

Work in animal systems suggests that transposons can be highly responsive to the distinction between somatic and germinal lineages. For instance, active versions of the *P* element of Drosphila are produced only in the germ line due to tissue-specific splicing of the *P* element transcript (Laski et al., 1986). Similarly, cytotype-specific negative regulation is apparently achieved at least in part via tissue-specific expression of repressor *P* elements (Misra and Rio, 1990). Also, *tom* and *gypsy*, two Drosophila retrotransposons, are highly expressed in the ovaries and appear to be transmitted via extracellular particles to the progeny (Kim et al., 1994; Tanda et al., 1994; Smith and Corces, 1995). In each of these cases, the level of functional transposase is enhanced or suppressed in particular tissues. This tissue-specific regulation is consistent with the idea that these mobile genetic elements have evolved the capacity to optimize transmission and minimize the damaging effects of unrestricted transposition.

Plant transposable elements have long been thought to be developmentally regulated. Based on her observations of the maize transposon *Suppressor-mutator* (*Spm*), McClintock (1961) suggested that transposable elements can respond to developmentally regulated signals. This regulation results in specific spatial and temporal patterns of transposon activity (McClintock, 1958). More recently, it has been demonstrated that these changes in activity, which can be both programmatic and heritable, are correlated with changes in the level of methylation in the subterminal repeats of *Spm* (Banks et al., 1988; Banks and Fedoroff, 1989). Because the germ line in plants is not sequestered, as it is in animals, these changes in activity during development can result in changes in the heritable copy number of *Spm* (McClintock, 1958; Fedoroff and Banks, 1988). However, it does not appear that *Spm* distinguishes specifically between terminally differentiated "somatic" and meiotic or immediately premeiotic "germinal" lineages.

The *Mutator* (*Mu*) system is composed of a heterogeneous family of transposable elements; all members of the family contain homologous terminal inverted repeats of ~200 bp, and each class is defined by its unique internal sequence (for

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reviews, see Walbot, 1991; Chandler and Hardeman, 1992; Bennetzen et al., 1993). The system is regulated by the *MuDR* class of elements (Chomet et al., 1991; Hershberger et al., 1991; Qin et al., 1991; James et al., 1993). Autonomous *MuDR* elements contain two open reading frames encoding transcripts of 2.8 kb (*mudrA*) and 0.9 kb (*mudrB*), both of which are associated with *Mu* activity (Chomet et al., 1991; Hershberger et al., 1995). These transcripts encode two proteins, MURA and MURB, respectively. The *Mu* system is highly mutagenic and capable of maintaining high levels of activity through multiple generations due to the high transposition frequency of both its autonomous and nonautonomous components (Alleman and Freeling, 1986; Lisch and Freeling, 1994).

The *Mu* transposable elements of maize appear to display pronounced developmental regulation. *Mu* elements are progressively modified epigenetically during development (Martienssen et al., 1990; Martienssen and Baron, 1994), and deletions of *MuDR* elements occur throughout development (Lisch et al., 1995). These age-dependent alterations correlate with male/female–specific differences in transmission efficiency of *Mu* activity (Walbot, 1986; Brown and Sundaresan, 1992). In addition, somatic excision events (as assayed by the appearance of revertant sectors) occur characteristically late in development and almost exclusively in the last few somatic cell divisions. This is in contrast to *Activator* (*Ac*) and *Spm*, which condition both late and early excisions (Levy and Walbot, 1990). Finally, somatic excisions and germinally transmitted duplications are quite frequent, but germinal transmission of excision events is exceedingly rare. Thus, it appears as though *Mu* is capable of making a distinction between "germinal" and "somatic" lineages, unlike other plant transposable elements.

Here, we provide evidence that the MURB protein is localized in a tissue-specific manner. We found that MURB is detected at high levels during much of male inflorescence development but is absent in the immediate progenitors of the pollen. Based on these observations, we discuss the relevance of this pattern of expression to the developmental regulation of the *Mu* system as a whole. We also present a model that incorporates a number of aspects of *Mu* element behavior and regulation.

RESULTS

Generation of Antibodies Specific to MURB

We generated and purified polyclonal antibodies raised against bacterially expressed MURB. These antibodies recognize a 30-kD protein in maize tissue that segregates with the regulatory transposon *MuDR*. Shoot tissue, including all tissue above the root, was collected from six separate families derived from the same lineage (see Methods). The seedlings ranged in age from 3 to 8 weeks. DNA from leaf tissue from these plants was examined by DNA gel blotting for the presence of a 4.7-kb EcoNI fragment diagnostic of the *MuDR* regulatory element (data not shown), and the proteins were examined by either immunoblotting or in situ immunolocalization for the presence of the 30-kD protein. This protein was expressed in all 36 *MuDR*$^+$ individuals (plants carrying one or more *MuDR* elements) examined and was not expressed in any of the 25 *MuDR*$^-$ individuals (plants lacking any *MuDR* elements). An immunoblot of protein extracts from two representative individuals from each class is shown in Figure 1. Based on these data, we conclude that the 30-kD protein detected in maize tissue is the *MuDR*-encoded MURB protein.

The predicted molecular mass of MURB is 23 kD (Hershberger et al., 1995). Because we detected a 30-kD protein, this suggests that MURB may be post-translationally modified. Comparison of the predicted amino acid sequence with known post-translational modification sequences in the GenBank data bank reveals homologies with several known glycosylation and phosphorylation sequences (data not shown). These putative modifications could be functionally important in the regulation of *Mu* activity and may account for the discrepancy between the observed and predicted molecular masses. These modifications could also account for the presence of the additional, slightly smaller protein visible in many of the *MuDR*-containing extracts assayed (Figure 1).

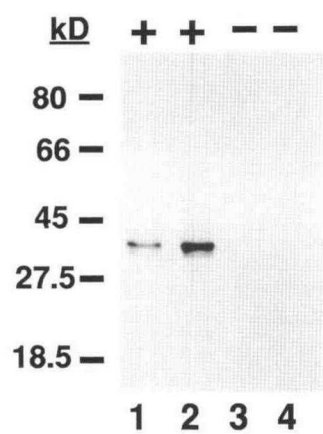

Figure 1. Characterization of Anti-MURB Antibodies Using Immunoblot Analysis.

Maize protein extracts were fractionated by SDS-PAGE on a 12% SDS–polyacrylamide gel and transferred to PVDF membrane for immunoblot analysis. Lanes 1 and 2 are designated (+) and contain protein extracts from 10-week-old and 12-week-old ear tissue from *MuDR*$^+$ plants. Lanes 3 and 4 are designated (−) and contain protein extracts from 10-week-old and 12-week-old ear tissue from *MuDR*$^-$ plants. The numbers at the left indicate mass values in kilodaltons (kD) of a molecular mass standard. The immunoblot was incubated with purified anti-MURB antibodies.

Immunolocalization of MURB during Male Inflorescence Development

Because there are such large differences between the transmission characteristics of *Mu* element duplications and *Mu* element excisions, we focused our efforts on male floral development, from vegetative apical meristem through anther development. We used immunolocalization with anti-MURB antibodies to look for patterns of localization that might relate to the nature of *Mu* developmental regulation.

We first examined shoot apical meristems from 3-week-old seedlings. Figures 2A through 2C show median longitudinal sections of shoot apical meristem. *MuDR*$^+$ individuals were probed with either the anti-MURB antibodies (Figure 2A) or the anti-ubiquitin antibodies (Figure 2C), and a *MuDR*$^-$ section was probed with the anti-MURB antibodies (Figure 2B). MURB is detected primarily in the leaf primordia, coinciding with those cells that are actively dividing. However, MURB is not detected in the meristem itself and is variably detected in the ground tissue and the oldest leaf tissue. For comparison, no signal was observed in the *MuDR*$^-$ tissue probed with the anti-MURB antibodies (Figure 2B), whereas ubiquitin was detected at similar levels in all cells and tissue types of the section (Figure 2C).

Because they represent an early stage in male flower development, staminate tassel inflorescences from 4-week-old related *MuDR*$^+$ individuals were probed with the anti-MURB antibodies (Figure 2D) or the anti-ubiquitin antibodies (Figure 2E). MURB was detected primarily in the developing spikelet primordia. MURB was detected at the highest level in the most actively dividing cells of the inflorescence. MURB was not excluded from the ground cells of the inflorescence, but it appeared to be present at a much lower level in those cells. In contrast, ubiquitin was present at similar levels in all cells of the inflorescence.

As the staminate inflorescence develops, MURB continues to be localized in tissues that contain actively dividing cells. Figures 3A through 3F depict sections of 5-week-old developing *MuDR*$^+$ tassel spikelet tissue that were probed with the anti-MURB antibodies (Figures 3A, 3C, and 3E) or the anti-ubiquitin antibodies (Figures 3B and 3D). In Figure 3F, *MuDR*$^-$ tissue was probed with the anti-MURB antibodies. The results clearly show that MURB is localized to the tissue that is differentiating into florets, whereas ubiquitin expression is uniform throughout the spikelet. One particularly striking example of this specificity is evident in Figure 3E. In this section, it appears that MURB is specifically localized to the outer four layers of the spikelet primordium. We suggest that this is only evident in some of these longitudinal sections because MURB is only absent in the innermost layers of the spikelet primordia.

Because they represent a somewhat later stage of floral development, tassel spikelets in which the florets had begun to differentiate were examined next. Figures 3G through 3I compare sections of 6-week-old tassel spikelets that show the developing floret pair from two related individuals. The *MuDR*$^+$ individual was probed with the anti-MURB antibodies (Figure 3G), and the *MuDR*$^-$ individual was probed with the anti-ubiquitin antibodies (Figure 3H) and the anti-MURB antibodies (Figure 3I). At this stage of development, MURB expression is localized to the developing florets and to the surrounding lemma and glume tissue. The *MuDR*$^-$ tissue probed with the anti-MURB antibodies (Figure 3I) shows no signal and confirms that the MURB is present only in *MuDR*$^+$ tissue.

Finally, we examined floral tissue in which anthers were nearly fully formed but in which pollen had not yet differentiated. Figure 4 shows tissue sections from a 7- to 8-week-old *MuDR*$^+$ individual probed with the anti-MURB antibodies (Figures 4A and 4D) or the anti-ubiquitin antibodies (Figure 4B) and from a related *MuDR*$^-$ individual of the same age probed with the anti-MURB antibodies (Figure 4C). These sections show the anther, including the innermost sporogenous tissue surrounded by three layers of anther wall tissue, and a final epidermal cell layer (Kiesselbach, 1949). The cells of the sporogenous tissue are the microspore mother cells or their immediate progenitors. These are the cells that give rise to the male gametes. They are closely related to the tapetal cells of the inner anther walls but only distantly related to the outermost epidermal cells of the anther (Dawe and Freeling, 1990). MURB was detected specifically in the epidermis and all anther cell wall layers but not in the innermost sporogenous tissue (Figures 4A and 4D). This differential detection of MURB is not an artifact of staining, as adjacent sections probed with the anti-ubiquitin antibodies (Figure 4B) clearly show that the tissue and proteins in the innermost layer are intact and that specific proteins can be readily detected by immunolocalization.

MURB is neither confined to nor excluded from the nucleus, but as the tissue became more differentiated, MURB was detected prominently within the nucleus. No known nuclear localization signals were apparent in the primary amino acid sequence of MURB. However, MURB is small enough to potentially cross the nuclear envelope without a targeting sequence (Peters, 1986). It is also possible that MURB could form a complex with the larger *MuDR*-encoded protein, MURA, whose sequence contains two putative nuclear targeting signals (Hershberger et al., 1991) and hence may be transported into the nucleus with MURA.

DISCUSSION

MURB Immunolocalization

We generated antibodies specific to MURB, one of the two proteins encoded by *MuDR*, the regulatory transposon of the *Mu* transposable element family of maize. Immunolocalizations using these antibodies were performed on tissues at various developmental stages and revealed a tissue-specific pattern of accumulation of MURB that also changes with the

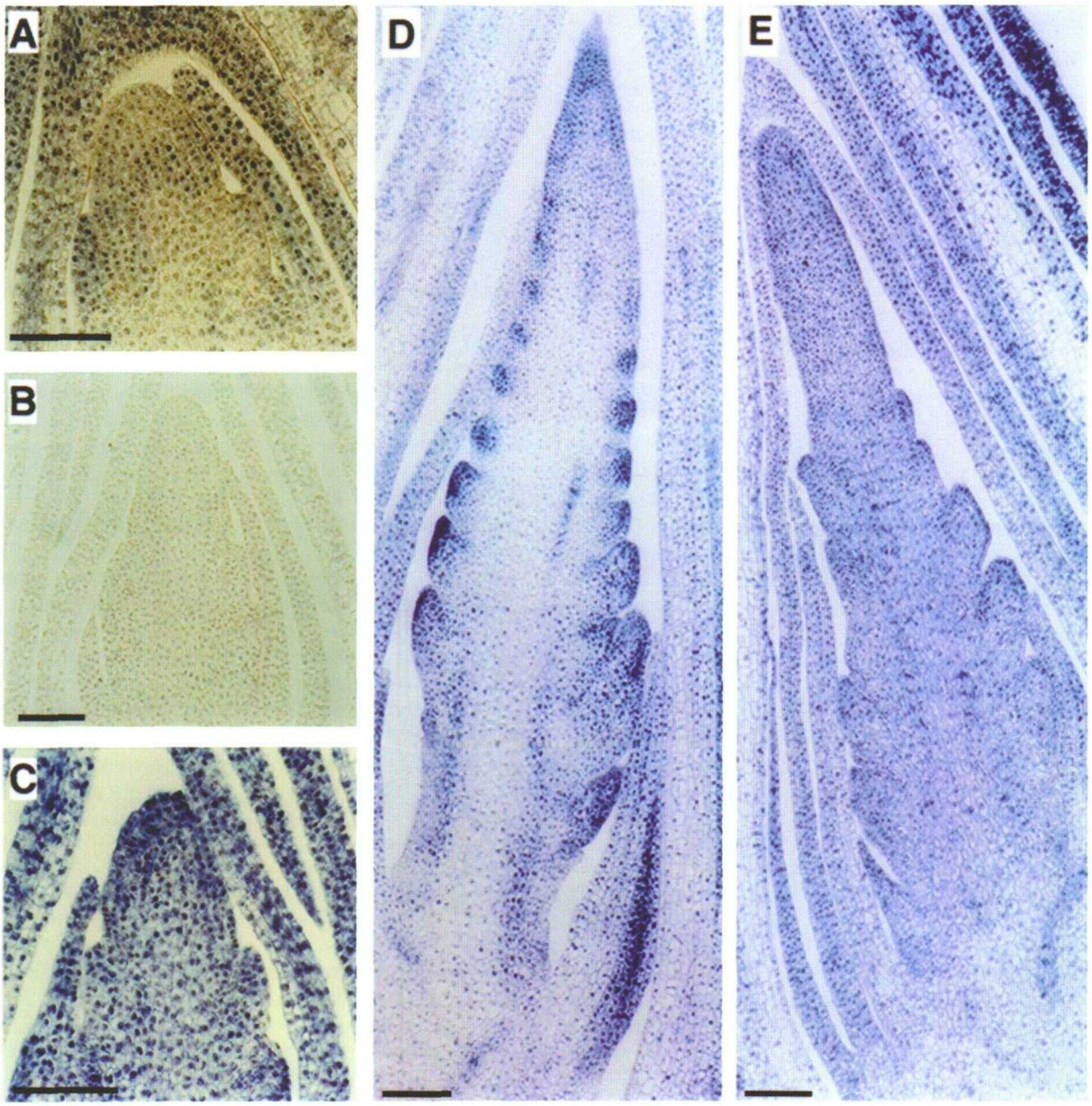

Figure 2. Immunolocalization of MURB and Ubiquitin in Vegetative Meristems and Inflorescence Meristems.

Vegetative meristems from 4-week-old (**[A]** to **[C]**) and 5-week-old (**[D]** and **[E]**) maize seedlings were fixed in formaldehyde, embedded in paraffin, and sectioned. Immunolocalizations were then performed. Scale bars in **(A)** to **(E)** = 100 μm.

(A) *MuDR*$^+$ meristem probed with purified anti-MURB antibodies.

(B) *MuDR*$^-$ meristem probed with purified anti-MURB antibodies.

(C) *MuDR*$^+$ meristem probed with anti-ubiquitin antibodies.

(D) *MuDR*$^+$ inflorescence meristem probed with purified anti-MURB antibodies.

(E) *MuDR*$^+$ inflorescence meristem probed with anti-ubiquitin antibodies.

developmental age of cell lineages. Actively dividing cells outside of the vegetative meristem contain this protein, whereas cells in the meristem itself do not. This is apparent in leaf (Figure 2) and inflorescence (Figures 2 and 3) primordia. MURB was detected at very high levels in the male inflorescence primordium and throughout most of the development of the male inflorescence; MURB is most abundant in those lineages that may be included in the germ line (Figure 3). However, we did not observe MURB in the cells of the sporogenous tissue of developing anthers (Figure 4). This tissue includes the

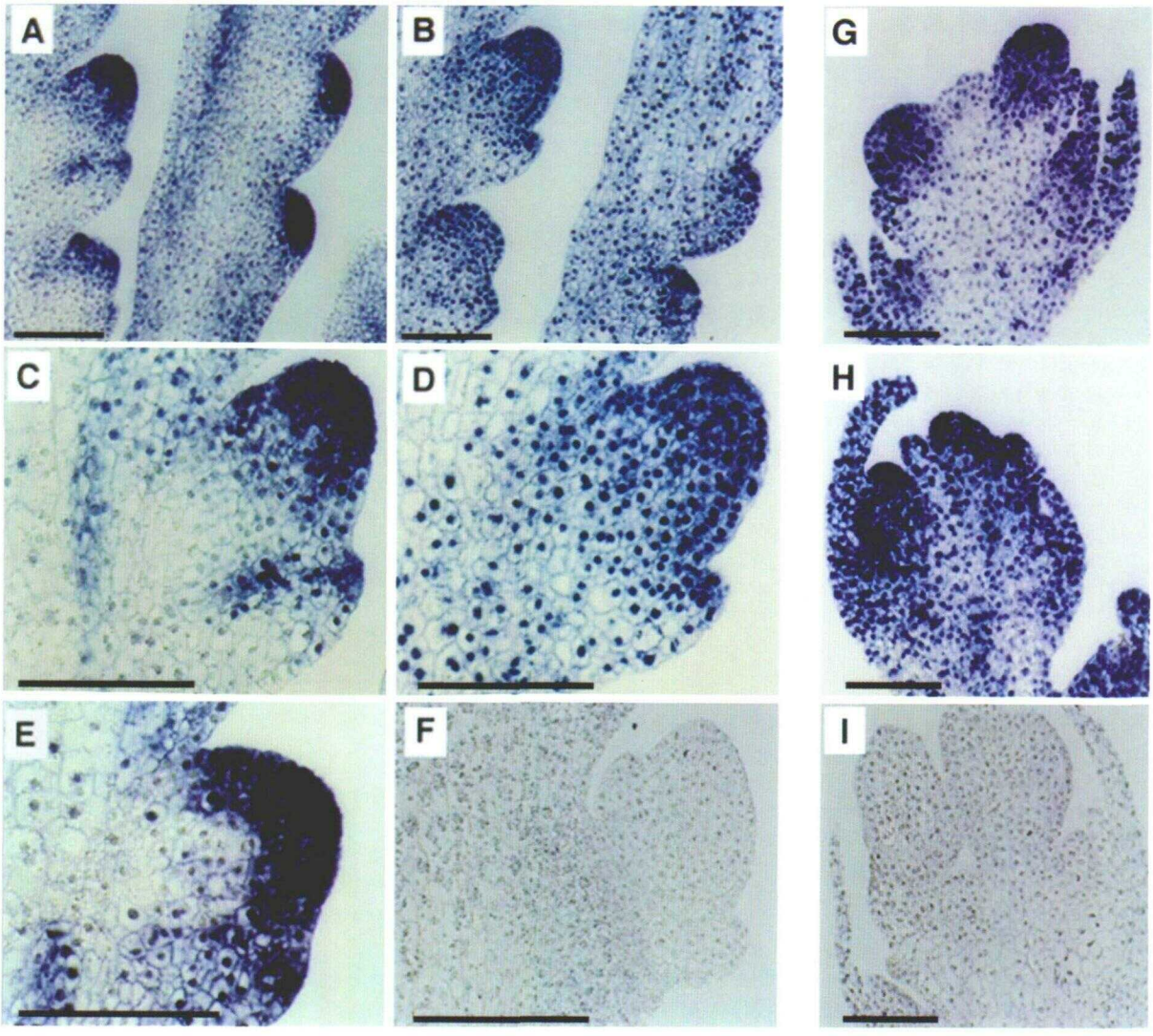

Figure 3. Immunolocalization of MURB and Ubiquitin in Tassel Spikelet and Floret Primordia.

Tassel spikelet primordia (**[A]** to **[F]**) from 5-week-old plants and floret primordia (**[G]** to **[I]**) from 6-week-old maize plants were fixed in formaldehyde, embedded in paraffin, and sectioned. Immunolocalizations were then performed. Scale bars in **(A)** to **(I)** = 100 μm.

(A) *MuDR*$^+$ spikelet primordia probed with purified anti-MURB antibodies and magnified.

(B) *MuDR*$^+$ spikelet primordia probed with anti-ubiquitin antibodies and magnified.

(C) Same sample as shown in **(A)** at a higher level of magnification.

(D) Same sample as shown in **(B)** at a higher level of magnification.

(E) A more precisely medial section of a spikelet primordium from the same tassel as shown in **(A)**.

(F) A *MuDR*$^-$ spikelet primordium probed with purified anti-MURB antibodies.

(G) *MuDR*$^+$ floret primordia probed with purified anti-MURB antibodies.

(H) *MuDR*$^-$ floret primordia probed with anti-ubiquitin antibodies.

(I) *MuDR*$^-$ floret primordia probed with purified anti-MURB antibodies.

microspore mother cells, and it is these cells that will undergo meiosis and give rise to the pollen.

The function of MURB is not known. Hence, it would be premature to offer a single explanation for these observations. However, the pattern of expression of this transposon-encoded protein is clearly related to development, so it is reasonable to discuss its function in the context of the relationship between *Mu* activity and maize development. There are two aspects of the developmental regulation of *Mu* activity that we wish to consider in relation to the observed pattern of MURB

expression: (1) the almost exclusively late somatic reversion events and (2) the extremely low frequency of germinal revertants.

Late Somatic Reversions

Mu-associated somatic reversions occur only very late during development, but *Mu* activities, such as transposition, can occur throughout development. Note that somatic reversion is not synonymous with *Mu* element transposition: we have shown previously that duplications of a specific nonautonomous *Mu* element are not associated with excision of that *Mu* element from its original position (Lisch et al., 1995). In addition, we have observed numerous examples of duplications of and deletions within a *MuDR* element at various times during development (Lisch et al., 1995). These results demonstrate that it is only the reversion events that are restricted to the last few cell divisions during somatic development. Therefore, it appears that *Mu* activities are developmentally regulated such that there is a shift late in somatic development from duplication to reversion events.

MURB is less abundant in those somatic cells that are not actively dividing. If "lateness" in somatic tissue is associated with reduced mitotic activity, then the reduced level of MURB may be related to the shift from "early" somatic development to "late" somatic development. Therefore, one potential relationship between the pattern of MURB localization and the observed *Mu* activities may be that in somatic tissue, a large amount of MURB is required for new insertions of *Mu* elements, but a reduced amount results in excision of *Mu* elements.

It is also possible that another factor, such as MURA, is specifically active only late during development, and it is this factor, either alone or in combination with a reduced level of MURB, that is responsible for the appearance of late reversions. This hypothesis is supported by our observation that deletions within *MuDR* elements disrupting MURA but not MURB result in the loss of somatic excision events (Lisch and Freeling, 1994). However, if MURA is only on late during development, this suggests that *Mu* element duplication, which occurs only in the presence of *MuDR* elements, does not require that protein. We think this is a highly unlikely scenario because the *mudrA* transcript is always associated with active *MuDR* elements (Qin and Ellingboe, 1990; Chomet et al., 1991; Hershberger et al., 1995). Furthermore, because the promoters for the *mudrA* and *mudrB* genes are nearly identical (Hershberger et al., 1995), it is a distinct possibility that the two genes are expressed in a similar manner.

However, other levels of regulation are certainly possible. There may be changes in splicing efficiency of the *MuDR* transcripts as development proceeds, or these *MuDR*-encoded proteins may undergo post-translational modifications that alter their functions such that only excisions can occur. In any event, the shift from one mode of activity (transpositions associated with duplications) to the other (excisions associated with reversions) is clearly correlated with a decrease in the amount of MURB late in development.

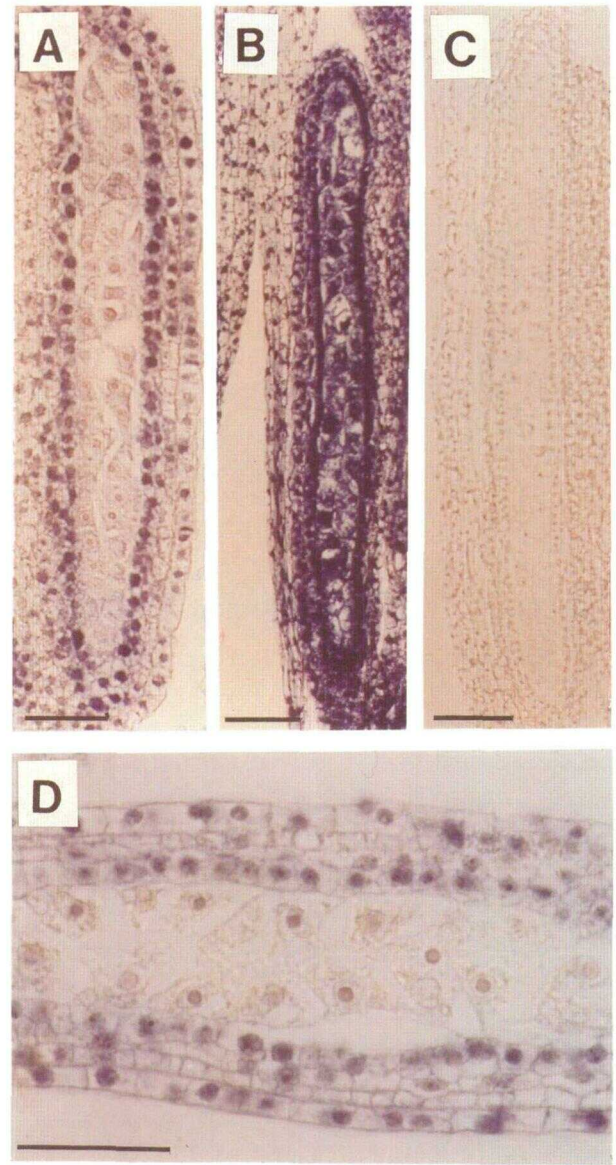

Figure 4. Immunolocalization of MURB and Ubiquitin in Anther Tissue.

Anthers from 7- to 8-week-old maize plants were fixed in formaldehyde, embedded in paraffin, and sectioned. Immunolocalizations were then performed. Scale bars in **(A)** to **(D)** = 50 μm.

(A) *MuDR*$^+$ anther tissue probed with purified anti-MURB antibodies.
(B) *MuDR*$^+$ anther tissue probed with anti-ubiquitin antibodies.
(C) *MuDR*$^-$ anther tissue probed with anti-MURB antibodies.
(D) *MuDR*$^+$ anther tissue probed with purified anti-MURB antibodies.

Low Germinal Reversion Frequency

The other aspect of the developmental regulation of *Mu* activity that may be related to MURB expression is the lack of germinally transmitted reversions of *Mu*-induced mutations. As germinally transmitted duplications of *Mu* elements occur at frequencies approaching 100% (Alleman and Freeling, 1986), the rarity of these reversion events is not due simply to a lack of germinal activity. In addition, there is no evidence that the appearance of large numbers of newly inserted *Mu* elements subsequent to outcrossing of plants carrying active *Mu* elements is associated with the concomitant loss of any *Mu* elements originally present in those plants (Alleman and Freeling, 1986; Lisch et al., 1995). These observations demonstrate that although excisions of *Mu* elements are actively excluded from germinal lineages in the plant, duplications of *Mu* elements are not.

Differentiation of the cell lineages in maize that are specifically germinal occurs early in the development of each loculus of the anther. A single column of cells in the developing stamen gives rise to both the inner wall of the locule and the pollen mother cells (Kiesselbach, 1949); it is these cells that undergo meiosis and give rise to the pollen. MURB is most highly expressed in the actively dividing cells that immediately precede the differentiation of the microspore mother cells, but it is conspicuously absent in the microspore mother cells themselves. Excision events are excluded in these cells; therefore, it seems likely that the loss of MURB and the lack of excisions are linked. This linkage could be purely coincidental, but it is reasonable to hypothesize that *MuDR* downregulation in the microspore mother cells (as reflected by the loss of MURB) is causally related to the lack of germinal reversion events.

Gap Repair and *Mu* Element Transposition

The observed regulation of MURB lends itself to some interesting mechanistic possibilities. One such possibility is that *Mu* elements transpose via a double-stranded gap repair mechanism (reviewed in Lowe et al., 1992; Lisch et al., 1995) similar to that used by *P* elements of Drosophila. When *P* elements excise, a double-stranded gap is produced. That gap is thought to be repaired using either the sister chromatid or the homologous chromosome as a template (Engels et al., 1990). If the sister chromosome is used as a repair template, the result of the original excision event can be an apparent duplication of the original element. Interruptions of this repair process have been suggested to result in the production of *P* element deletion derivatives (Kurkulos et al., 1994). If the homologous chromosome is used as a repair template, the result is an apparent precise excision event. If the *P* element was originally inserted into a gene resulting in a loss of function and if the homologous chromosome carries a wild-type copy of the gene, the result of this repair is a reversion of the mutant phenotype. When the homolog is no longer present, the result is a drastic reduction in the number of reversion events. Those few reversion events that remain may be the result of a repair pathway that uses the host sequence duplication that had been created upon the original insertion of the *P* element (Engels et al., 1990).

Like *P* elements, *Mu* elements appear to transpose duplicatively (Alleman and Freeling, 1986; Lisch et al., 1995). Unlike *P* elements, however, a high frequency of somatic reversion of *Mu* element–induced mutations does not require the presence of nonmutant sequences on the homolog (Doseff et al., 1991; D. Lisch, unpublished data). Therefore, if *Mu* elements do use a gap repair mechanism to achieve duplication, somatic reversion is not the result of repair using the homolog. Nevertheless, *Mu* element footprints are consistent with the production of double-stranded gaps and with template-based repair pathways (Britt and Walbot, 1991; Doseff et al., 1991).

A Differential Gap Repair Model

If we assume that *Mu* elements employ a gap repair mechanism, it is possible to construct a model that correlates well with all of the observations (Figure 5). We call this model the "differential gap repair model." According to this hypothesis, *Mu* elements excise at various times during development. The outcome of this initial excision event depends on template availability, which we hypothesize can vary, depending on the cell type in which the excision occurred. In actively dividing cells, such as those in the spikelet primordia depicted in Figure 4, the double-stranded gaps produced by *Mu* element excision would be repaired using the sister chromatid as a template. The result of this repair process would be the apparent duplication of the *Mu* element, which is frequently observed in these cells. If the repair process were interrupted before completion, the result would be the loss of those internal sequences not yet synthesized; this phenomenon is also frequently observed in somatic sectors (Lisch et al., 1995). Because homologous chromosomes do not pair in these somatic cells, the homologous chromosome may not be readily accessible for template-mediated repair. Therefore, according to this model, excisions of *Mu* elements from mutant genes are not prevented from occurring early in somatic development. However, because the subsequent gap repair is hypothesized to use only the sister chromatid, those excision events would not restore gene function.

Because cells terminally differentiate late during somatic development, we hypothesize that repair shifts from a template-mediated process to a mechanism involving simple ligation of the double-stranded breaks. As has been suggested for *P* elements (Engels et al., 1990), these repairs could be mediated by pairing 5′ overhangs left at the duplicated original insertion site after *Mu* element excision. This shift may be the result of the reduced level of MURB in these cells, or it may arise from an increase in the level of another factor such as

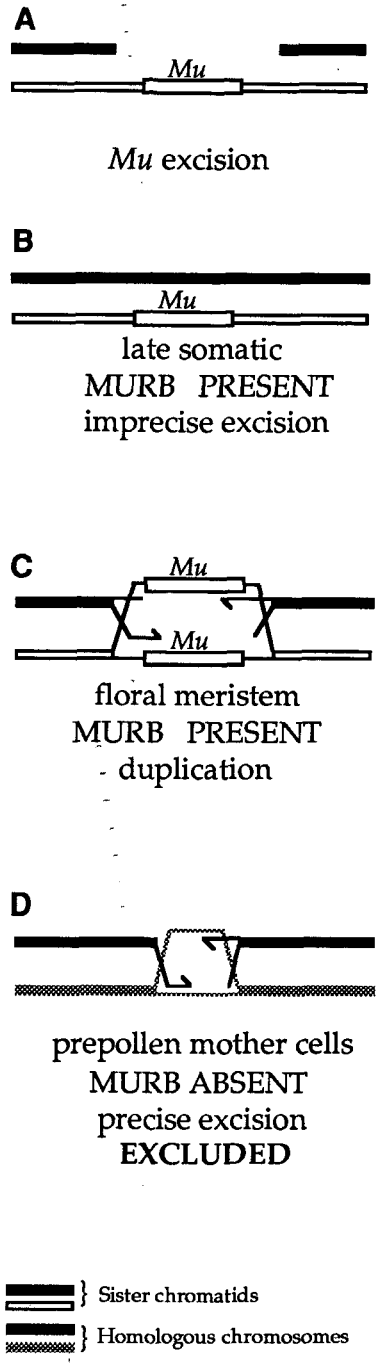

Figure 5. A Gap-Repair–Based Model for the Developmental Regulation of *MuDR* Activity.

(A) Double-stranded breaks are created by *Mu* element excision.
(B) These gaps may be repaired without the use of either the sister chromatid or the homologous chromosome as a template.
(C) and **(D)** Alternatively, the gaps may be repaired using either the sister chromatid or the homologous chromosome, respectively.
The tissues in which these pathways are hypothesized to occur and the consequence of each are as indicated.

MURA. Alternatively, the shift may reflect a change in the host cell's repair mechanism for broken chromosomes, or *Mu* elements may be excising before DNA replication so that the sister is unavailable for use as a template in these cells.

In the above scenario, gap repair in actively dividing somatic cells uses the sister chromatid as a template because the homologous chromosomes are less accessible. However, in cells undergoing meiosis, the homologous chromosome must be accessible, because they are involved in recombination and hence may be available for *Mu*-induced repair as well. If *Mu* elements employ a gap repair pathway and if they were capable of actively transposing in cells undergoing meiosis, one would expect to observe repair using the homologous chromosome. That repair would be expected to result in germinally transmitted precise excision events. However, these excision events are quite rare. If MURB expression is indicative of *Mu* activity in general, then we suggest that the lack of germinal revertants may result from the loss of activity specifically in those cells in which the consequence of that activity would be homolog-mediated repair.

It is important to emphasize that loss of *Mu* activity specifically in microspore mother cells would not result in the loss of germinally transmitted duplications. Activity is high earlier in development (Figures 2 and 3) of the male inflorescence, when repair is hypothesized to use the sister chromatid as a template (Lisch et al., 1995). In addition, *Mu*-induced mutations have often been observed to occur after meiosis during the mitotic development of the gametophyte. These insertion events result in nonconcordant kernels in which the embryo and the endosperm differ genetically (Robertson and Stinard, 1993). Thus, insertions of *Mu* elements can occur immediately before or immediately after meiosis. Our model suggests that it is only in a very few, specialized cells that activity must be eliminated to prevent the production of precise excisions resulting from repair using the homologous chromosome.

We suggest that the observed variations in MURB expression may be a mechanism for maximizing the heritable copy number of *Mu* elements. Reinsertion of *Mu* elements is likely to be <100% efficient. In those cells in which repair is from the sister chromatid, a failure to reinsert would have no effect on *Mu* element copy number; successful reinsertions in these cells at any frequency would result in a net increase in copy number. However, the result of homolog-mediated gap repair would produce a net reduction in copy number. A variant of *MuDR* capable of selective deactivation of transposase function in those cells in which the homolog is used as a template would have an advantage over *Mu* variants that remained active in those cells. Therefore, we suggest that the observed loss of MURB in the microspore mother cells may represent a mechanism to maximize *Mu* copy number by minimizing the loss of *Mu* elements through homolog-mediated gap repair.

It is possible that MURB is not observed in the microspore mother cells for other reasons as well. Perhaps MURB expression reduces the viability of the pollen mother cells to such an extent that there has been strong selection against it. Conversely, it is not proved that MURB is absolutely required for

Mu element transposition. Therefore, the presence or absence of this protein may be irrelevant to the activity of *Mu* elements. However, the transcript encoding this protein is invariably present in fully active *Mu* lines (Qin and Ellingboe, 1990; Chomet et al., 1991; Hershberger et al., 1995), and the accumulation of MURB appears to be tightly regulated; thus, specific functions are likely.

The differential gap repair model makes a number of predictions. First, as germinally transmitted precise excisions are rare regardless of whether plants are crossed as males or as females, we expect to see a loss of MURB in the female megaspore mother cells, analogous with the loss of this protein in the microspore mother cells. The model also predicts that the loss of MURB in those cells is representative of the loss of *Mu* activity in general, suggesting that MURA should also be missing in the microspore mother cells. Finally, it should be possible to obtain a mutation (either in *MuDR* itself or in a host factor) that would allow MURB (and/or MURA) to accumulate in the microspore mother cells. Such a mutation would result in a very large increase in the frequency of germinally transmitted reversion events. These predictions are under investigation.

An exploration of the mechanism by which tissue specificity of *MuDR* expression is achieved should shed light on both the transposon and its host. If *MuDR* is responding to basic developmental cues, then this transposon may be uniquely useful in understanding both transposon regulation and plant development.

METHODS

Maize Stocks

The *a1-mum2* allele was originally isolated by D.S. Robertson, and the line containing the *a1-mum2* in this study was obtained from S. Dellaporta (Yale University, New Haven, CT). *MuDR* is the regulator of the *Mutator* (*Mu*) system of transposable elements. Kernels carrying *MuDR* show small red clonal sectors on a pale yellow background. In the absence of *MuDR*, the kernel is pale yellow. The *a1-sh2* tester line lacking *MuDR* activity was obtained from B. McClintock. Progeny segregating for the *MuDR* element(s) were obtained from the cross *MuDR*$^+$; *a1-mum2 Sh2/a1 sh2* × *a1 sh2/a1 sh2*; *MuDR*$^-$ or *MuDR*$^+$; *a1-mum2 Sh2/a1 sh2* × *a1-mum2 Sh2/a1 sh2*; *MuDR*$^-$.

DNA Analysis

Maize leaf DNA was isolated according to Cocciolone and Cone (1993) from the leaf tissue of each individual that had been subjected to either protein gel blot or in situ immunolocalization analysis. A plasmid containing an *MuDR* deletion derivative inserted into the *Sh2* gene has been described previously by Chomet et al. (1991). The EcoRI-BamHI fragment of this deletion derivative, designated Mu*, was used as a probe for detection of the presence of *MuDR* (Chomet et al., 1991). *MuDR* was detected as a 4.7-kb fragment when Mu* was used to probe EcoNI-digested DNA. DNA gel blots were performed according to Lisch et al. (1995).

Mutator Clones

A cDNA clone (cDNA-B2) containing the coding sequence for all but the two N-terminal amino acids of the smaller of the two *MuDR*-encoded proteins, MURB, was obtained from K. Hardeman and V. Chandler (University of Oregon, Eugene, OR; Hershberger et al., 1995). The coding sequence for amino acids 12 to 154 from a total of 207 amino acids was cloned into pGEX-3X (Pharmacia) to make a fusion between the MURB and glutathione-*S*-transferase (GST) open reading frames as follows: the *mudrB* cDNA was digested with AlwNI and XbaI. A 426-bp fragment corresponding to nucleotides 4440 to 3946 of the spliced *mudrB* cDNA was isolated. The infrequently spliced third intron was not included (Hershberger et al., 1995). The ends were filled in with T4 DNA polymerase (Promega), and this fragment was ligated into pGEX-3X that had been digested with EcoRI and blunt ended with T4 DNA polymerase. This expression clone was designated pGX(MURB).

Antibody Production

pGX(MURB) was transformed into *Escherichia coli* BL21 (Hanahan, 1983), and the fusion protein was expressed at 37°C for 3 hr after induction with 0.4 mM isopropyl-β-D-thiogalactopyanoside. Cells were collected by centrifugation and lysed for 20 min at room temperature with 1 mg/mL of lysozyme in 50 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 150 mM NaCl, 10% sucrose, 1 mM DTT, 10 mM β-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride. The remaining steps were performed at 4°C unless otherwise noted. Sodium deoxycholate was added to a final concentration of 0.1%, and the lysate was incubated for 15 min with stirring. The lysate was sonicated to disrupt the DNA and was centrifuged at 12,000 rpm for 20 min. Triton X-100 was added to the supernatant (25 mL) to a final concentration of 1%; 500 μL of a 50% slurry of glutathione–Sepharose (Pharmacia) in 50 mM Tris-HCl, pH 8, was added, and the lysate was incubated with the resin for 1 hr with gentle agitation. The resin was pelleted by centrifugation at 2800 rpm for 5 min, the supernatant was removed, and the resin was washed with two changes of 20 mL of phosphate-buffered saline (PBS; 130 mM NaCl, 7 mM Na_2HPO_4, and 3 mM NaH_2PO_4). The MURB–GST fusion protein was eluted from the resin with 5 mL of 10 mM reduced glutathione (Sigma) in 50 mM Tris-HCl, pH 8. The protein was concentrated from 5 mL to 250 μL in an Amicon-10 spin concentrator (Amicon, Beverly, MA). The fusion protein was judged to be >90% pure by SDS-PAGE.

To generate antibodies to the MURB–GST fusion, two rabbits were injected with 1 mg of purified fusion protein suspended in adjuvant (MPL + TDM + CWS emulsion; RIBI ImmunoChem Research, Hamilton, MT) and were subsequently boosted in an identical manner three times at 4-week intervals. Serum was collected 7 to 10 days after each injection. A response to the GST–MURB fusion protein could be detected on immunoblots after the first boost. However, no proteins specific to *MuDR* plants could be detected on immunoblots of crude protein extracts from plants carrying *MuDR*, but rather, numerous cross-reacting bands were visible.

Preparation of Affinity Columns

To allow detection of *MuDR*-specific proteins, the antibodies were affinity purified. The MURB–GST fusion protein was cross-linked to

glutathione–Sepharose by using a heterobifunctional cross-linker sulfo-SMCC (Pierce, Rockford, IL). Two milliliters of a 50% slurry of glutathione–Sepharose was equilibrated in 100 mM $NaPO_4$, pH 7.5. This was added to 2 mg of sulfo-SMCC, and the resulting mixture was incubated for 1 hr at 30°C with constant agitation. Excess sulfo-SMCC was removed by centrifugation, and the resin was equilibrated in 100 mM $NaPO_4$, pH 6.5. Three milligrams of the purified MURB–GST fusion protein was equilibrated in 100 mM $NaPO_4$, pH 6.5, and was mixed with the activated GST–Sepharose resin for 16 hr at 4°C with gentle agitation. Unconjugated protein was removed by centrifugation, and the resin was suspended in 50 mM Tris, pH 7.5, and packed into a column.

To remove antibodies specific for GST, an affinity column of GST was also prepared. One milliliter of a 50% slurry of Reacti-gel-6X (Pierce) was used in a reaction with 3 mg of purified GST in 50 mM borate, pH 8.6, according to the manufacturer's instructions. The GST affinity resin was equilibrated in 50 mM Tris, pH 7.5, before purification of the anti-MURB antibodies.

Purification of Anti-MURB Antibodies

Proteins in the crude sera were precipitated by the addition of an equal volume saturated $(NH_3)SO_4$, suspended in 50 mM Tris-HCl, pH 7.5, and dialyzed against 50 mM Tris-HCl, pH 7.5. The serum was depleted of anti-GST antibodies on a GST–agarose column equilibrated in 50 mM Tris-HCl, pH 7.5. The anti-MURB antibodies were affinity purified by binding to a MURB–GST Sepharose column equilibrated in 50 mM Tris-HCl, pH 7.5. Nonspecifically bound antibodies were removed with 1 M guanidine-HCl in 50 mM Tris-HCl, pH 7.5, and anti-MURB antibodies were eluted with 4.5 M $MgCl_2$ in 50 mM Tris-HCl, pH 7.5, containing 1 mg/mL of BSA. The fractions containing anti-MURB antibodies were dialyzed against PBS with 40% glycerol and stored at −20°C.

Protein Extracts from Maize Tissue

Crude protein extracts of young leaf tissue were obtained from 0.4 g tissue (fresh or frozen) homogenized in 2 mL of buffer containing 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 10% sucrose, 40 mM β-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride. The extracts were filtered through glass wool, the insoluble matter was pelleted in a microcentrifuge, and the protein content of the supernatant was determined using the bicotinic acid assay (Pierce). The extracts were frozen in liquid N_2 and stored at −70°C. Samples were prepared for SDS-PAGE by adding one-fifth of the volume sample buffer containing 0.25 M Tris-HCl, pH 6.8, 10% β-mercaptoethanol, 5% SDS, and 50% glycerol.

Immunoblot Analysis

Maize protein extracts were fractionated by SDS-PAGE using 12% gels and transferred to polyvinylidine fluoride membrane for immunoblot analysis. Membranes were blocked with 3% nonfat dry milk in PBS-0.2% Tween 20 followed by overnight incubation with the primary antibodies (1:1000) in PBS-0.2% Tween-20 at 4°C. The antigen–antibody complex was detected by incubation for 2 hr with goat anti-rabbit alkaline-phosphatase–conjugated secondary antibodies (Boehringer Mannheim) diluted 1:6000 PBS-0.2% Tween 20. The complex was visualized with nitro blue tetrazolium/5-bromo 4-chloro 3-indoylphosphate (NBT/BCIP) in 100 mM Tris-Cl, pH 9.5, 100 mM NaCl, and 50 mM $MgCl_2$.

Tissue Fixation

Tissue was dissected and fixed in formaldehyde according to Jackson (1991). Fixed tissue was dehydrated in an ethanol series, infiltrated with Histoclear (National Diagnostics, Manville, NJ), and embedded in paraffin (Paraplast Plus, Monoject Scientific, St. Louis, MO). Sections (8 μm) were cut from the paraffin blocks on a rotary microtome and mounted on poly-lysine–coated Probe-On Plus slides (Fisher Scientific, Pittsburgh, PA).

Immunolocalizations

Tissue sections were dewaxed in Histoclear, transferred to 100% ethanol, and rehydrated in a graded ethanol and water series. The sections were treated for 10 min at room temperature with proteinase K (Bethesda Research Laboratories) at 50 to 150 mg/mL in PBS. The amount of proteinase K used was optimized for each tissue type and each lot of proteinase K. The sections were rinsed with PBS and then incubated in a blocking solution of 2% nonfat dry milk in PBS-0.05% Tween-20 for 1 hr at room temperature.

Purified anti-MURB antibodies or anti-ubiquitin antibodies (Sigma) were diluted 1:100 in the blocking solution, and 30 mL was placed on each slide. The slides were covered to prevent dehydration and were incubated overnight at 4°C. The remaining steps were performed at room temperature. The tissue sections were washed with three changes of PBS-0.05% Tween-20 with gentle agitation for 10 to 15 min; they were then incubated for 2 hr with goat anti–rabbit alkaline-phosphatase–conjugated secondary antibodies (Boehringer Mannheim) diluted 1:600 in the same buffer. The sections were washed as before, equilibrated with 100 mM Tris, pH 9.5, 100 mM NaCl, and 50 mM $MgCl_2$ and visualized using NBT/BCIP for 2 to 20 hr. Following color development, the slides were dehydrated and mounted for microscopy using Merckoglas (EM Science, Wakefield, RI). The slides were examined on a Zeiss Axiophot microscope (Zeiss, Thornwood, NY) and photographed using Kodak Royal Gold 100 color print film (Eastman Kodak, Rochester, NY). For Figures 1 through 5, photographs were scanned and digitized using a Microtek ScannMaker II digital scanner (Microteck, Redondo Beach, CA), and color balance and size were adjusted using Adobe Photoshop 2.5 (Adobe Systems Inc., Mountain View, CA). Composite figures were printed using a Tektronix Phaser 440 dye sublimation printer (Tektronix Inc., Wilsonville, OR).

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