Planta (1998) 205: 547–552

Planta


# Dormancy-associated gene expression in pea axillary buds.

## Cloning and expression of PsDRM1 and PsDRM2

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Received: 10 December 1997 / Accepted: 23 January 1998

**Abstract.** Pea (*Pisum sativum* L. cv. Alaska) axillary buds can be stimulated to cycle between dormant and growing states. Dormant buds synthesize unique proteins and are as metabolically active as growing buds. Two cDNAs, PsDRM1 and PsDRM2, were isolated from a dormant bud library. The deduced amino acid sequence of PsDRM1 (111 residues) is 75% identical to that of an auxin-repressed strawberry clone. PsDRM2 encodes a putative protein containing 129 residues, which includes 11 repeats of the sequence [G]-GGGY[H][N] (the bracketed residues may be absent). PsDRM2 is related to cold- and ABA-stimulated clones from alfalfa. Decapitating the terminal bud rapidly stimulates dormant axillary buds to begin growing. The abundance of PsDRM1 mRNA in axillary buds declines 20-fold within 6 h of decapitation; it quickly reaccumulates when buds become dormant again. The level of PsDRM2 mRNA is about three fold lower in growing buds than in dormant buds. Expression of PsDRM1 is enhanced in other non-growing organs (roots $\gg$root apices; fully-elongated stems $>$elongating stems), and thus is an excellent "dormancy" marker. In contrast, PsDRM2 expression is not dormancy-associated in other organs.

**Key words:** Apical dominance – Axillary bud – Bud development – Dormancy (bud) – *Pisum* (bud dormancy)

### Introduction

Leaf axils typically contain one or more vegetative buds. While these buds have the potential to develop into growing shoots, most buds remain dormant throughout the life of the plant. Dormancy, broadly defined, is the temporary arrest of visible growth of a plant structure that is capable of growing; inhibition of axillary bud growth by the terminal bud is called apical paradormancy or, more commonly, apical dominance (Lang 1987). Dormancy processes allow plants and plant organs to survive under adverse conditions or, in the case of apical dominance, to be held in reserve in the event that the terminal bud is lost or damaged (Stafstrom 1995). Dormant organs can resume growing when environmental or endogenous conditions change. Although this developmental plasticity is critical to plant survival, little is known about the molecular regulation of bud dormancy in any plant (Dennis 1994; Lang 1994).

Apical dominance was one of the first developmental phenomena shown to be regulated by a plant hormone (reviewed in Tamas 1995). Thimann and Skoog used *Vicia faba* and Alaska pea plants to demonstrate that auxin derived from the terminal bud inhibits the growth of axillary buds (Skoog and Thimann 1934; Thimann and Skoog 1934). Based on the polarity of auxin transport, it is likely that auxin inhibits axillary buds indirectly (Snow 1937; Morris 1977). Indirect auxin action also is consistent with more recent findings. For example, growing *Phaseolus* buds contain more auxin than dormant buds (Gocal et al. 1991) and auxin-responsive genes are expressed preferentially in growing pea buds (Stafstrom 1993). Cytokinins promote axillary bud development even in the presence of auxin (Wickson and Thimann 1958). In *Cicer*, axillary buds begin to grow within a few hours of decapitating the terminal bud; the abundance of cytokinins increases rapidly within these buds as they begin to grow (Turnbull et al. 1997). The amount of ABA is somewhat higher in dormant buds than growing buds of *Phaseolus* and *Elytrigia* (Gocal et al. 1991; Pearce et al. 1995), which together with other evidence suggests a role for this hormone in inhibiting bud growth (Tamas 1995).

Node-2 of Alaska pea plants contains four dormant buds, all of which begin to grow following decapitation of the terminal bud (Stafstrom and Sussex 1992). After 2–3 d, rapid growth by the largest bud (bud-1) inhibits further growth of the smaller buds, which become

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dormant again. Removing bud-1 stimulates the smaller buds to resume growing (bud-2, the second largest bud, and bud-1 are studied here). Thus, the growing and dormant states can be readily manipulated and more than one complete "growth-dormancy cycle" can be completed in just a few days.

The dormancy-to-growth transition occurs very rapidly in pea axillary buds. Visible growth of bud-1 is detected within 8 h of decapitating intact plants (Stafstrom and Sussex 1992). Increased expression of genes associated with cell proliferation (cdc2 kinase, MAP kinase, and histones H2A and H4) and other growth-associated genes (ribosomal proteins L27 and L34) is detected in these buds within 1 h of decapitation (Devitt and Stafstrom 1995). Furthermore, in situ hybridization analysis shows that increased accumulation of ribosomal protein L27 (RPL27) mRNA occurs in all regions of the bud within one h (Stafstrom and Sussex 1992). In *Populus* buds, expression of cdc2 kinase and cyclin genes also is correlated with the growing state (Rohde et al. 1997).

Dormancy-associated proteins decline in abundance within 3 h of decapitating the terminal bud (Stafstrom and Sussex 1988; Stafstrom 1993). In addition, dormant buds incorporate radiolabeled amino acids into proteins at rates equivalent to or greater than growing buds, indicating that dormant buds are metabolically active (Stafstrom and Sussex 1988). Therefore, we consider dormancy to be an active developmental state. Cloning and expression of dormancy-associated genes in axillary buds has not been described previously. Here we describe the isolation of PsDRM1 and PsDRM2 cDNA clones. Expression patterns of mRNAs corresponding to these clones were analyzed in axillary buds, growing and non-growing stems and roots, floral organs and leaves. PsDRM1 expression was tightly linked to the non-growing developmental state in all organs tested.

## Materials and methods

*Plant material*. Pea seeds (*Pisum sativum* L. cv. Alaska) were imbibed overnight in running tap water, sown in trays containing Promix and grown in a greenhouse. Ambient temperature ranged from about 20 to 30 °C, depending on the season. Natural light was supplemented with artificial light to maintain a 16-h light/8-h dark photoperiod. Node-2 of intact 7-d-old plants contains four dormant axillary buds. The two largest buds, bud-1 and bud-2, measure approximately 2.0 mm and 0.7 mm, respectively. Decapitating the terminal bud of these plants directly above node-2 stimulates all dormant buds to begin growing. Bud-1 was collected at several time points during the ensuing 24 h (dormancy-to-growth transition). Bud-2 was collected daily through 6 d after decapitation; after 5 d (stage 5), bud-1 was removed, which stimulated bud-2 to resume growing (stage 5 + 1). The dormancy-to-growth transition in bud-2 was studied by collecting buds at intervals between stage 5 and stage 5 + 1. Buds and other organs were frozen in liquid nitrogen and stored at −80 °C.

Growing (elongating) stem tissue was isolated from internode-5 of 10 d-old pea plants. This internode, which measured 7 mm or less at the time of collection, typically would have elongated to about 100 mm. Mature (fully-elongated) stem tissue was from internode-3 of 10-d-old plants. Fully-expanded leaflets were from leaves at node-3 or node-4 of 10-d-old plants. Floral organs (sepals, petals, stamens and carpels) were from flowers 1 d prior to pollen anthesis, a stage that was easy to recognize and occurred about 21 d after sowing under our growth conditions. Root apices (terminal 2 mm, including root cap) were isolated from 3-d-old seedlings sown on moist paper towels. Fully-elongated root tissue (10 and 20 mm from the apex) was collected from the same seedlings.

*Isolation of dormant bud-associated cDNA clones*. Total RNA was purified from dormant axillary buds collected from intact 7-d-old pea plants. Polyadenylated RNA was isolated using the PolytractAT kit (Promega, Madison, Wis., USA) and used to construct a directional cDNA library in the pSPORT1 plasmid vector (Gibco-BRL, Gaithersburg, Md., USA). The resulting library, which contained about 2 million primary recombinants, was amplified once. Colonies were transferred to 96-well microtiter plates. Replica filters were screened by differential hybridization using dormant-bud and growing-bud cDNA probes. Approximately 1500 colonies were screened. Seven putative dormancy-associated clones were analyzed on Northern blots containing total RNA. Of these, three were found to be growth-associated, two could not be detected and the remaining two, called PsDRM1 and PsDRM2, are described in this report. Nested deletion clones were generated using the Erase-A-Base kit (Promega). These clones were sequenced manually (Sequenase II kit; US Biochemical, Cleveland, Ohio, USA) and using an Applied Biosystems PRISM 373 automated sequencer (Biotechnology Core Facility at Northern Illinois University).

*RNA gel blotting*. Total RNA was isolated from buds and other tissues using extraction with guanidinium isothiocyanate and phenol followed by LiCl precipitation (Stafstrom and Sussex 1992). The RNA (10 μg/lane) was separated by denaturing formaldehyde gel electrophoresis and blotted onto nylon membranes by capillary transfer. Random-primed $^{32}$P-labeled probes were prepared from gel-purified cDNA inserts (DecaPrime II; Ambion, Austin, Tex., USA). Blots were prehybridized in 50% formamide, 5× Denhardt's reagent (1 × Denhardt's = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), 0.5% SDS, 5× SSPE (1 × SSPE = 150 mM NaCl, 10 mM $Na_2PO_4$, 2 mM EDTA, pH 7.4) and 100 μg/mL salmon sperm DNA for 2 h at 42 °C, and hybridized overnight under the same conditions. Blots were washed in 0.2 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M $Na_3$-citrate, pH 7) and 0.1% SDS at 55 °C and exposed to X-ray film. Some blots were stripped and reprobed up to two times. Patterns of PsDRM1 and PsDRM2 mRNA accumulation were compared to those of RPL27, a growth-associated marker (Stafstrom and Sussex 1992). RNA loadings were assessed by ethidium bromide staining of rRNAs and by probing blots with BB695, which recognizes 28S rRNA (gift of L. Kaufman, University of Illinois, Chicago). Relative levels of mRNA accumulation were quantified from the original autoradiographs using a Hewlett-Packer ScanJet 3C scanner, and Photoshop and ImageCalc software.

## Results

*Isolation and sequence analysis of dormancy-associated cDNA clones*. A cDNA library corresponding to poly(A)$^+$ RNA from dormant pea axillary buds was screened for dormancy-associated clones. Two cDNAs, called PsDRM1 and PsDRM2, were found to be expressed preferentially in dormant buds. The complete sequences of these cDNAs were determined from sets of nested deletion clones. PsDRM1 is 554 base pairs (bp) in length (data not shown). PsDRM1 has an open reading frame that would encode a 111-amino-acid polypeptide (Fig. 1A). A BLAST sequence search (Altschul et al. 1990) identified λSAR5, an auxin-repressed cDNA from

A. PsDRM1

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PsDRM1   M--LDKLWDDIVAGPQPERGLEKLRKLTTTL--KDDGASNQLMRST   42
λSAR5    MVLLDKLWDDIVAGPQPERGLGMLRKVPQPLNLKDEGESSKI----
         *  ******************  ***.   *  **.* *...

PsDRM1   SIPTTPTTPVYPTTPSSARKVDNVWRSVFNPGSNSATKSIGAHVFD   88
λSAR5    TMPTTPTTPVTPTTPISARK-DNVWRSVFHPGSNLSSKTMGNQVFD
         ..******** **** **** ********.**** ..*..* .***

PsDRM1   KPLPNTPTVYDWMYSGDTRSKH-R                        111
λSAR5    SPQPNSPTVYDWMYSGETRSKHHR                       112
          * **.**********.***** *
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B. PsDRM2

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PsDRM2   MDSRKAMLILGLLAMVLLISSEVSARELTE-------EVVEKSDEV  39
CORA     MDSKKAILMLSLLAMAL-ISSVMSARDLTETSTDAKKEVVEKTNEV
GRPA                    MVLLISSEVSARDLTETTSDAKKEVVEKTNEV
         ***.**.*.*.**** * *** .***.***       ******.**

                  1     2     3     4     5     6
PsDRM2   NDAKYGGYGRGGGYHNGGGYHNGGGGYNGGGGYHNGGGGYNGGGGY  85
CORA     NDAKYGG.........[glycine-rich repeats]........
GRPA     NDAKYGG.........[glycine-rich repeats]........
         *******

           7      8      9      10     11
PsDRM2   HNGGGGYHNGGGGYNGGGGYHNGGGGYHGGGGHGGHGGASNNGN   129
CORA     .......[glycine-rich repeats].......QTEDNTQN   204
GRPA     .......[glycine-rich repeats].......QAKDNTQN   159
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**Fig. 1A,B.** Alignment of deduced amino acid sequences of PsDRM1 with λSAR5 from strawberry (Reddy and Poovaiah 1990) and PsDRM2 with alfalfa CORA and GRPA proteins (Luo et al. 1991; Laberge et al. 1993). **A** PsDRM1 encodes a putative protein of 111 amino acid residues that is 75% identical to λSAR5. **B** PsDRM2 encodes a putative glycine-rich protein containing 129 amino acid residues. Eleven copies of the glycine-rich repeat [G]GGGY[H][N] (bracketed residues may be absent) are numbered. The first 45 overlapping residues of PsDRM2 and CORA are 78% identical, and the first 32 overlapping residues of PsDRM2 and GRPA are 91% identical. Optimal sequence alignments were determined using Clustal V software. Gaps (-) and positions containing identical (*) and similar (•) amino acids are indicated. Genbank accession numbers for PsDRM1 and PsDRM2 are AF029242 and AF029243, respectively

strawberry receptacle (Reddy and Poovaiah 1990), as being similar to PsDRM1. The amino acid sequences encoded by these clones are identical at 79 of 106 overlapping positions (75%) and similar at 94 positions (89%). Most of the dissimilarity occurs between residues #26–42 of PsDRM1. This region also showed the greatest variability when either clone was compared to similar clones from apple (Lee et al. 1993; gb #L15194) or *Arabidopsis* (Newman et al. 1994; gb #T04159)(data not shown).

PsDRM2 is 466 bp in length and contains an open reading frame encoding a 129-amino-acid polypeptide (Fig. 1B). PsDRM2 encodes a putative glycine-rich protein that is similar to alfalfa clones encoding GRPA and CORA proteins, which are expressed in response to cold temperatures, drought stress and ABA (Luo et al. 1991; Laberge et al. 1993). The N-terminus of PsDRM2 shows a high level of identity with the alfalfa clones: 78% of the first 45 overlapping residues PsDRM2 and CORA are identical, and 91% of the first 32 overlapping residues of PsDRM2 and GRPA are identical. Each of the 3 proteins contains a series of repeat motifs that are rich in glycine, histidine, asparagine and tyrosine: PsDRM2 contains 11 tandem repeats of the sequence [G]GGGY[H][N] (the bracketed residues may be absent); CORA contains 7 copies of YNHGGG and 11 copies of HGG; and the sequence GGGYNHGGG-GYN is repeated 7 times in GRPA.

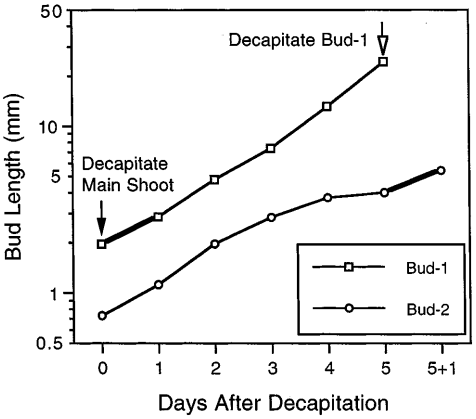


**Fig. 2.** Growth of bud-1 and bud-2 following decapitation of the terminal bud. On intact pea plants (day 0) bud-1 and bud-2 were dormant and measured about 2.0 and 0.7 mm, respectively. Decapitation of the terminal bud (*solid arrow*) stimulated these buds to begin growing. Rapid growth by bud-1 inhibited further growth of bud-2 beginning at about day 2. Decapitation of bud-1 on day 5 (*open arrow*) stimulated bud-2 to resume growing 1 d later (5 + 1 d). The thick lines indicate the 24 h dormancy-to-growth transition stages in bud-1 and bud-2 that were analyzed in Figs. 4 and 5. $n = 25$

*Growth-dormancy cycles in pea axillary buds.* Axillary buds on intact Alaska pea plants seldom develop into growing shoots. Following decapitation of the terminal bud, all four buds at node-2 began to grow. Only the largest and second largest bud at this node (bud-1 and bud-2, respectively) were studied here. Five days after decapitating the main shoot, bud-1 had grown in length from about 2 to 25 mm (Fig. 2). Bud-2 grew for the first 2 d and then, under the inhibitory influence of the rapidly growing bud-1, ceased growing and became dormant again. Bud-2 resumed growing after bud-1 was removed. In experiments described below, gene expression was analyzed: in bud-2 from 0 to 5 + 1 d stages, which includes greater than one complete growth-dormancy cycle (Fig. 3); and during 24 h dormancy-to-growth transitions in bud-1 and in bud-2 (Figs. 4, 5).

*Gene expression in axillary buds.* Dormant buds on intact plants contained relatively low amounts of RPL27 mRNA, a growth-associated marker (Fig. 3, 0 d). In contrast, these buds contained higher concentrations of PsDRM1 and PsDRM2 mRNAs. One day after decapitating the terminal bud, growing axillary buds contained several-fold more RPL27 mRNA, no detectable PsDRM1 mRNA and about one-third the amount of PsDRM2 mRNA as in dormant buds. At two days after decapitation, buds still contained a near-maximal amount of RPL27 message. PsDRM1 and PsDRM2 mRNAs had begun to increase in abundance at this stage. At 3, 4 and 5 days after decapitation, the concentrations of RPL27, PsDRM1 and PsDRM2 messages were similar to those found in dormant buds on intact plants (0 d). One day after removing bud-1 (5 + 1 days), bud-2 contained a high concentration of

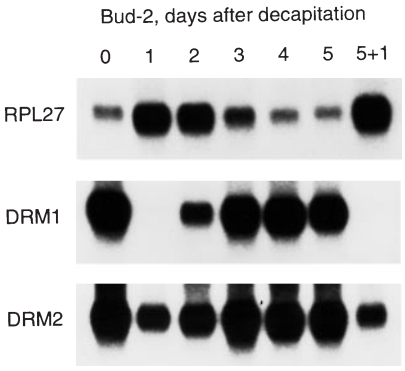


**Fig. 3.** Northern blot analysis of gene expression during growth-dormancy cycles in bud-2 following decapitation of pea plant. Buds were dormant on day 0, growing on days 1 and 2, dormant on days 3 to 5, and growing again on day 5 + 1 (see Fig. 2). RPL27 mRNA (≈600 bases) accumulated preferentially in growing buds (Stafstrom and Sussex 1992). PsDRM1 mRNA (≈600 bases) was abundant in dormant buds but undetectable in growing buds. PsDRM2 mRNA (≈800 bases) accumulated preferentially in dormant buds but also was present in growing buds. Total RNA (10 μg/lane) was isolated from bud-2 at the indicated number of days after decapitating the terminal bud. RNA was separated by denaturing formaldehyde gel electrophoresis and transferred to nylon membranes. The membranes were hybridized with $^{32}$P oligo-labeled probes corresponding to each cDNA clone

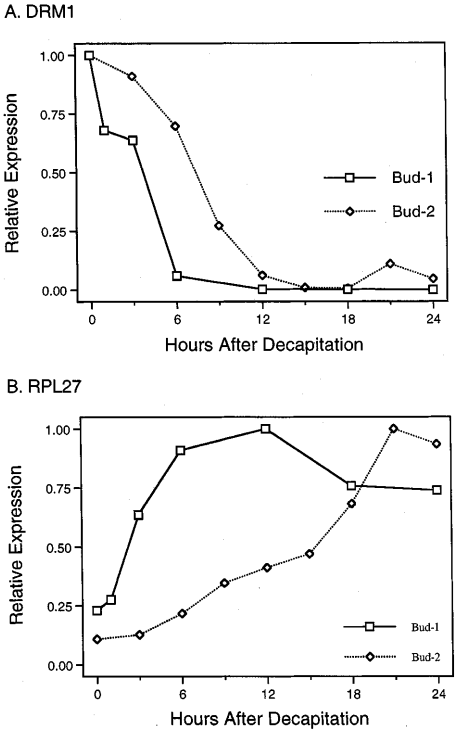


**Fig. 5A,B.** Relative amounts of RPL27 and PsDRM1 mRNAs in bud-1 and bud-2 during dormancy-to-growth transitions in decapitated pea plants. Relative mRNA accumulation was quantified by scanning the original autoradiographs. **A** The decline in abundance of PsDRM1 mRNA was more rapid in bud-1 than in bud-2. **B** RPL27 mRNA increased more rapidly in bud-1 than bud-2

RPL27 mRNA, no detectable PsDRM1 mRNA and a reduced amount of PsDRM2 mRNA.

The dormancy-to-growth transition was examined in bud-1 following decapitation of the terminal bud and in bud-2 after removing bud-1 (intervals denoted by thick lines in Fig. 2). Accumulation of RPL27 mRNA in bud-1 reached a maximum by six h after decapitation whereas maximal expression of this gene in bud-2 was not reached until at least 12 h after decapitation (Fig. 4). The PsDRM1 message in bud-1 had declined to a nearly undetectable level by 6 h after decapitation; a similar reduction in PsDRM1 expression in bud-2 was not seen until about 12 h after decapitation. In bud-2, PsDRM2 mRNA gradually declined in abundance and reached a minimum at about 15 h (PsDRM2 expression in bud-1 was not analyzed). PsDRM1 and RPL27 mRNAs were quantified by scanning the original autoradiographs (Fig. 5). PsDRM1 mRNA declined much more quickly in bud-1 than in bud-2, and RPL27 message increased more quickly in bud-1 than in bud-2. In bud-1, the content of PsDRM1 mRNA declined about 20-fold within 6 h of decapitation.

*Gene expression in other organs.* Accumulation of PsDRM1, PsDRM2 and RPL27 messages was assayed in other pea organs (Fig. 6). RPL27 mRNA accumulated preferentially in growing stems and root apices compared with their non-growing counterparts. In contrast, PsDRM1 accumulated preferentially in non-growing stems and roots. RPL27 was detected in leaflets and all floral organs but was abundant only in carpels. Moderate amounts of PsDRM1 message were present in sepals, stamens and carpels, but this message was barely detected in petals or leaflets. The pattern of PsDRM2 expression was considerably different from that of

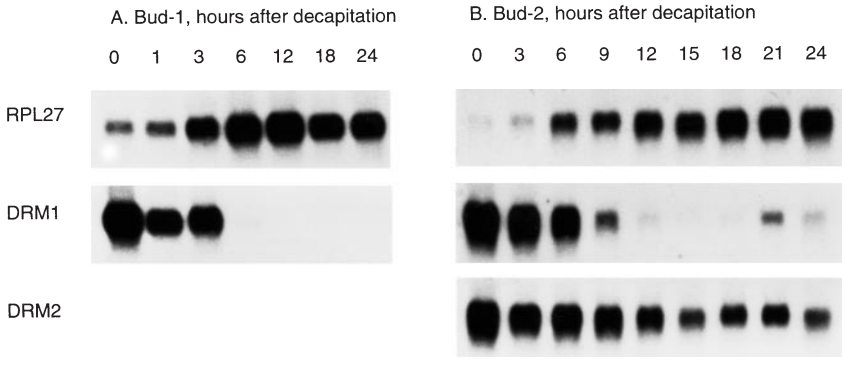


**Fig. 4A,B.** Northern blot analysis of gene expression during the dormancy-to-growth transition in bud-1 **A** and bud-2 **B** following decapitation of the terminal bud and removal of bud 1, respectively. Buds are dormant before decapitation (0 h) and growing by 24 h after decapitation (thick lines in Fig. 2). RPL27 mRNA is low in dormant buds and increases when buds begin to grow. PsDRM1 expression is reduced to undetectable levels in bud-1 within 6 h of decapitation; a similar reduction takes as long as 12 h to occur in bud-2. PsDRM2 expression also is linked to bud dormancy. Its expression in bud-2 declines about three fold over 24 h. Methods as for Fig. 3

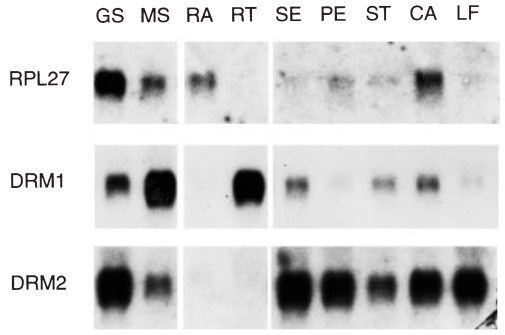


**Fig. 6.** Northern blot analysis of gene expression in other organs. RPL27 mRNA accumulated preferentially in growing stems and root apices (*GS*, *RA*) relative to their mature, non-growing counterparts (*MS*, *RT*). RPL27 mRNA was abundant in carpels (*CA*) and detectable in sepals, petals, stamens and leaflets (*SE*, *PE*, *ST* and *LF*, respectively). PsDRM1 mRNA was relatively abundant in mature stems and roots compared with growing stems and root apices. A moderate amount of PsDRM1 mRNA was present in sepals, stamens and carpels; this mRNA was barely detectable in leaves and petals. PsDRM2 mRNA was very abundant in growing stems and quite abundant in mature stems; none was detected in roots or root apices. Large amounts of PsDRM2 message accumulated in leaflets and all floral organs. Methods as for Fig. 3

PsDRM1 or RPL27. The concentration of PsDRM2 mRNA was higher in growing stems than in non-growing stems; this mRNA was not detected in roots or root apices. Leaflets and all floral organs contained relatively high levels of PsDRM2 mRNA.

## Discussion

The molecular biology of seed dormancy has been studied extensively and is moderately well understood (e.g., Bewley and Black 1994). By comparison, relatively little is known about molecular events associated with bud dormancy, including dormancy in over-wintering buds of woody perennials (Crabbé and Barnola 1996), tuber buds (Suttle 1996), and axillary buds inhibited by apical dominance. Pea axillary buds are highly amenable to study: hundreds of plants can be grown in a small area, plants are suitable for analysis when they are about a week old and, most importantly, growing and dormant developmental states can be interconverted quickly and repeatedly (Fig. 2). Increased expression of genes associated with organ growth and cell proliferation occurs within 1 h of decapitation (Stafstrom and Sussex 1992; Devitt and Stafstrom 1995). Therefore, dormant bud cells must be competent to respond rapidly to growth-promoting signals from the plant (Turnbull et al. 1997).

PsDRM1 and PsDRM2 were isolated from a dormant bud cDNA library by differential hybridization. Both mRNAs accumulated to high relative and absolute amounts in dormant buds (only short exposures to X-ray film were needed to detect these messages; Figs. 3,4). PsDRM1 is a much better marker for the dormant state than PsDRM2. The abundance of PsDRM1 mRNA decreased about 20-fold within 6 h of decapitation and this message was undetectable after 12 h (Figs. 4,5). This change in gene expression is as fast as any we have observed during dormancy-to-growth transitions in pea buds. In addition, accumulation of PsDRM1 in bud-2 appears to presage re-entry of growing buds into the dormant state. Based on the high level of expression of RPL27 in bud-2 at 2 d after decapitation, buds at this stage appeared to be in the growing state (Fig. 3). However, the presence of PsDRM1 message in these buds could indicate that they already had begun to revert to the dormant state.

PsDRM1 expression also is tightly linked to the non-growing or "mature" state in roots and stems (Fig. 6). For example, PsDRM1 message was not detected in root apices, which contain populations of rapidly dividing and elongating cells (Rost et al. 1988; Onelli et al. 1997), but was very abundant in mature root tissue, where cell division and elongation are absent or rare. Mature stems contained high levels of PsDRM1 mRNA, but this message also was detected in growing stems (Fig. 6). It is likely that growing stems contain a population of cells that is growing (dividing and elongating) and another population that has ceased growing. Similarly, sepals, stamens and carpels, which contain low levels of PsDRM1 message, may contain "growing" and "non-growing" cells. In-situ methods for detecting PsDRM1 mRNA, and perhaps PsDRM1 protein, might verify this conjecture. Since PsDRM1 is preferentially expressed in non-growing buds, stems and roots, it was surprising that fully-expanded leaflets from 10-d-old plants did not contain this message (Fig. 6). We plan to examine PsDRM1 expression in leaflets at this node from older plants and in leaflets from other nodes.

The amino acid sequence of PsDRM1 is very similar to that of λSAR5, an auxin-repressed mRNA from strawberry receptacle (Fig. 1; Reddy and Poovaiah 1990). Auxin-repression of PsDRM1 is consistent with what is known about the auxin content of axillary buds. For example, dormant axillary buds of *Phaseolus* contain less auxin than growing buds (Gocal et al. 1991). Furthermore, we have shown that two auxin-induced messages, pIAA4/5 and pIAA6, are more abundant in growing pea buds than in dormant buds (Stafstrom 1993). These results argue against the "direct theory" of auxin inhibition. PsDRM1 also shows some similarity to CRR48 (data not shown), a cytokinin-repressed cDNA isolated from cucumber cotyledons (Toyama et al. 1995). While increased concentrations of cytokinin (Turnbull et al. 1997) and auxin are probably involved in promoting bud growth, additional factors are likely to be involved (Beveridge et al. 1997). We are studying the regulation of PsDRM1 by hormones and other factors, which may lead to a better understanding of how apical dominance is controlled.

The temporal pattern of PsDRM2 mRNA accumulation is similar to that of PsDRM1, but a relatively large amount of PsDRM2 mRNA is present in growing buds (Figs. 3, 4). Since PsDRM2 is not expressed preferentially in other non-growing organs, it will have little value as a general "dormancy-marker". The glycine-rich alfalfa homologue of PsDRM2 is induced by ABA and by cold, drought, salinity and other stresses

(Luo et al 1991; Laberge et al. 1993). Dormant axillary buds typically contain somewhat higher levels of ABA than growing buds (Gocal et al. 1991; Pearce et al. 1995). It will be important to determine whether changes in PsDRM2 expression during growth-dormancy cycles (Fig. 3) occur in response to changing amounts of this hormone in pea buds.

Pea axillary buds are quite small, making it difficult to collect enough material for Northern blots and other experiments. Dormant bud-2 (5 d after decapitating the terminal bud) is more than twice as long and ten times as massive as dormant bud-1 from intact plants (Fig. 2). If the kinetics of the dormancy-to-growth transitions were similar in both buds, then bud-2 might be the preferred experimental system. We showed previously that cells in both dormant buds are arrested predominantly in G1 phase of the cell cycle (Devitt and Stafstrom 1995). Upon decapitation, bud-1 cells begin to enter S phase about 6 h after decapitation whereas bud-2 cells require at least 9 h. A similar delay in the onset of growth in bud-2 was apparent from the gene expression experiments described in this report. For example, it takes 9–12 h for PsDRM1 message to become undetectable in bud-2, whereas a similar level is reached in bud-1 between 3 and 6 h after decapitation (Figs. 4, 5). Nonetheless, bud-2 will continue to be useful for collecting dormant and growing buds when transition stages are not needed.

This work was supported by the Plant Molecular Biology Center, Northern Illinois University.

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