mentioned here) notably by Montgomery who is the leader of this progress. Other contributors are Huxley, Jutila and Ramachandra and others (For all these results see Titchmarsh[5] or Ivic[7]).

Prove or disprove (unconditionally) that

$$\frac{1}{T}\int_T^{2T} |\zeta(\tfrac{1}{2}+it)|^6 \, dt < T^{\varepsilon}, \tag{15}$$

for every $\varepsilon > 0$ and all $T \geq T_0(\varepsilon)$.

Note that eq. (15) is a consequence of LH which is a consequence of RH. Trivially, given the truth of eq. (15) with some (positive) exponent in place of 6, we can deduce its truth for all lower (positive real) exponents. So we look for the highest real power in place of 6 known today. This value is 4, due to Hardy and Littlewood (I do not know any proof of their result which avoids FE). The proof eq. (15) with 2 in place of 6 is easy and does not need FE. The following deep result due to Heath-Brown is certainly worthy of mention here (see ref. 5 or ref. 7). There holds

$$\frac{1}{T}\int_T^{2T} |\zeta(\tfrac{1}{2}+it)|^{12} \, dt < T^{1+\varepsilon}, \tag{16}$$

for every $\varepsilon > 0$ and all $T \geq T_0(\varepsilon)$.

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Role of *Bar* locus in development of legs and antenna in *Drosophila melanogaster*

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The X-linked *Bar* (*B*) mutation of *Drosophila melanogaster*, responsible for the well-known Bar eye phenotype due to over-expression of the BarH1 homeo-domain protein, is shown to enhance the abnormalities in legs and antennae of flies carrying a viable combination of certain *decapentaplegic* (*dpp*) loss of function mutant alleles. It is also shown that the homeo-domain carrying BarH1/BarH2 protein products of the *B* locus are expressed in a characteristic annular pattern in areas of normal larval leg and antennal discs that correspond to the distal regions of adult fly appendages. *dpp*-mutant background partly disrupts the expression pattern of Bar homeo-proteins in these discs and a combination of *B* and *dpp*-mutant alleles disrupts the Bar expression patterns in these imaginal discs much more severely. This is in agreement with the more severe phenotypes of legs and antennae of such flies. We suggest that the homeo-box containing *B* genes function as new members of the proximal distal sector genes and are important for patterning these appendages along their proximo-distal axes.

THE Bar eye mutant phenotype of *Drosophila melanogaster* is associated with a tandem duplication (*Bar* duplication) of the 16A1-7 region of the X chromosome[1], and is characterized by a drastically reduced number of ommatidia in the compound eyes of adult flies[2]. Organization of the *B* locus is complex since it harbours at least two homeo-box containing genes, the *BarH1* and *BarH2*, of which *BarH1* is reported to be over-expressed due to the *Bar* duplication[3,4]. The *decapentaplegic*, *dpp*, gene product is a member of the TGFβ family[5] and has very important roles in morphogenesis in many developmental pathways in *Drosophila*. The gene *dpp* is expressed in the eye discs of third-instar larvae of *Drosophila* in the anteriorly moving morphogenetic furrow and this is responsible for induction of differentiation of the precursor cells into ommatidia[6]. Over-expression of BarH1 homeoprotein in eye discs of *B* mutant larvae is associated with attenuation of *dpp* gene expression in the morphogenetic furrow[7]. As a result, ommatidial precursor cells fail to differentiate and instead, undergo apoptotic death. Consequently, the number of ommatidia in adult eyes of *B* mutant flies is substantially reduced[7]. All other adult structures are

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normal in the *B* mutant flies. Sato *et al.*[8] recently reported that *BarH1* and *BarH2* genes are expressed in the anterior-most notal region (prescutum) of the third-instar larval wing discs. Other than this, nothing is so far known about the expression and/or role of *B* genes in other larval imaginal discs.

During the course of our studies on interaction of *B* and *dpp* genes in developing eye discs, we screened several *dpp* mutants and found that a heteroallelic combination of *dpp*-recessive lethal alleles[9], viz. dpp^{d6} and dpp^{d12}, was viable. However, the dpp^{d6}/dpp^{d12} flies showed reduced number of ommatidia in their eyes, similar to that in the *B* mutant flies (unpublished). As expected from the wide roles of *dpp* in development and differentiation[10,11], the dpp^{d6}/dpp^{d12} flies were weak, short-lived (2–3 days), and displayed abnormalities in wings, legs, antennae, external genitalia, etc. During these studies, we further found that in the presence of the *B* mutant gene, the dpp^{d6}/dpp^{d12} heteroallelic combination resulted in total absence of ommatidia and, surprisingly, much more severely affected legs, antennae, etc. Since the *Bar* locus has so far not been reported to have any role in development and differentiation of these appendages, the intensification of abnormalities in the appendages in *B*; dpp^{d6}/dpp^{d12} flies was unexpected. Therefore, we examined expression of Bar proteins in developing leg and antennal imaginal discs in larvae of various genotypes, and the results are presented here. Our present work shows that besides the earlier known expression in the eye and the wing discs, the *B* genes are indeed expressed in a characteristic pattern in the leg and the antennal discs. On the basis of our results, we suggest that the homeo-box-containing *B* genes interact with *dpp* and function as new members of the proximal–distal sector genes, which are important for patterning these appendages along their proximo-distal axes.

Figure 1 shows the morphology of the prothoracic leg and antenna in wild-type (+/+), *B*, B^+; dpp^{d6}/dpp^{d12} and *B*; dpp^{d6}/dpp^{d12} flies. All the three pairs of legs in *Bar*-mutant flies (Figure 1 *b*) were indistinguishable from those in wild-type flies (Figure 1 *a*). In B^+; dpp^{d6}/dpp^{d12} flies on the other hand, the tarsal and meta-tarsal segments of all legs were affected due to loss of claws and fusion of the tarsal segments. On some occasions the dorsal parts were ventralized leading to loss of certain structures and duplication of others; the tibia and femur segments were progressively less affected while the trochanter and coxa were almost as in wild-type or *B* mutant flies (Figure 1 *c*). Most interestingly, in *B*; dpp^{d6}/dpp^{d12} flies, the severity of these abnormalities was

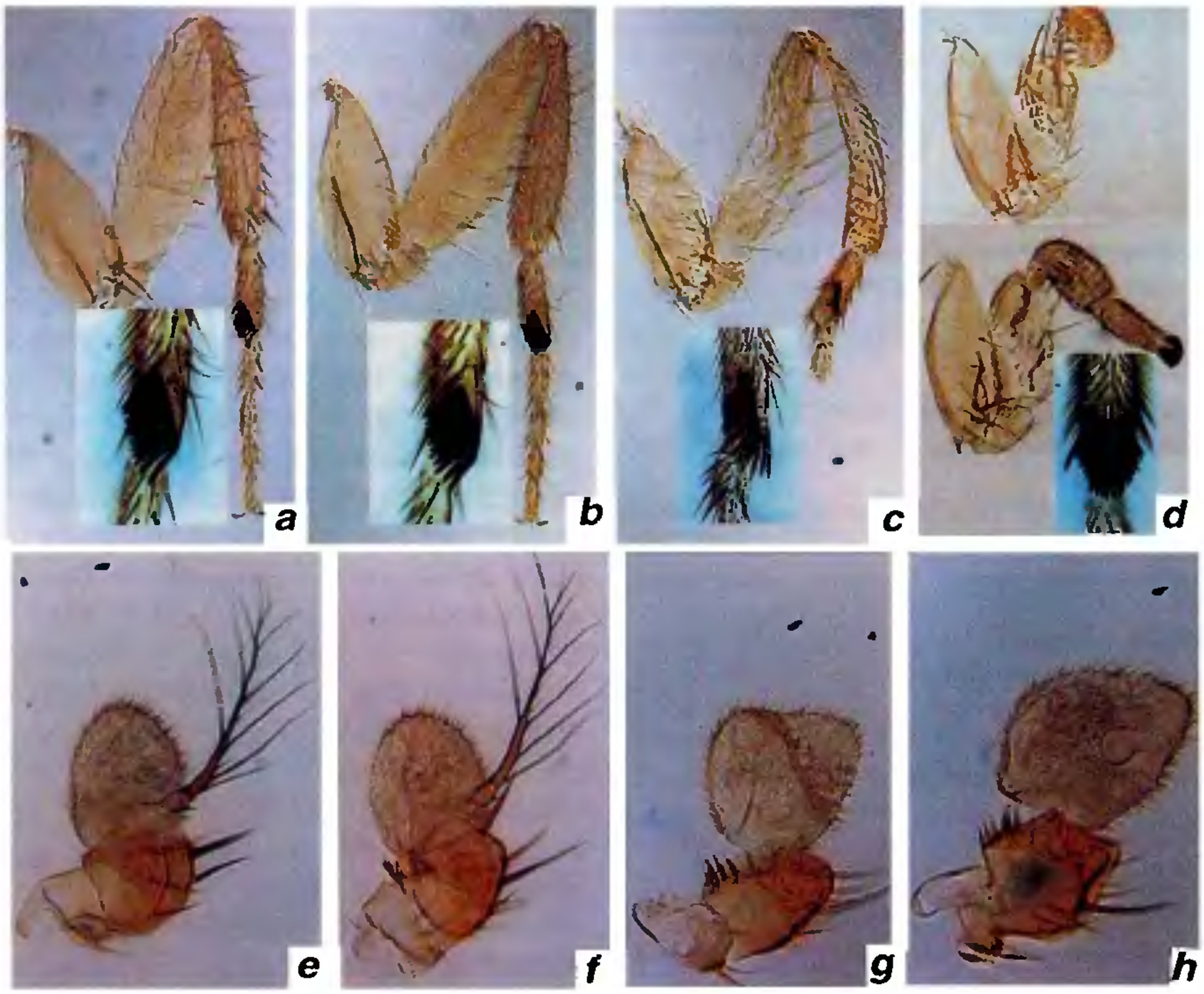

Figure 1. Prothoracic legs (*a–d*) and antennae (*e–h*) of wild-type (*a*, *e*) *B* (*b*, *f*), B^+; dpp^{d6}/dpp^{d12} (*c*, *g*) and *B*; dpp^{d6}/dpp^{d12} (*d*, *h*) male flies. Legs from two different flies are shown in *d*. The inset in each case is an enlarged view of the tarsal segment to show the sex-comb in the corresponding genotype.

further intensified. As evident from the two examples in Figure 1 *d*, there was an overall shortening in length along the proximal-distal axis accompanied by complete or partial fusion or even total loss of the tarsal segments. Moreover, the tibia and femur were also further deformed due to shortening, bulging and disorganized bristle patterns, although the most proximal segments like the coxa and the trochanter were not so much affected. Such extensive abnormalities in distal segments of appendages were never seen in B^+; dpp^{d6}/dpp^{d12} flies. The sex comb on tarsal segment of the prothoracic leg (shown as insets in Figures 1 *a–d*) of male flies was not affected by *B* mutation alone (compare the insets in Figure 1 *a* and *b*). However, in B^+; dpp^{d6}/dpp^{d12} male flies, the number of bristles in sex comb was generally more than in wild-type or *B* male flies (inset in 1 *c*). On the other hand, in *B*; dpp^{d6}/dpp^{d12} male flies, the sex comb, when present, was always duplicated (Figure 1 *d* and inset).

In the antennae also, while the *B* mutation by itself did not result in any abnormality (compare Figure 1 *e* and *f*), B^+; dpp^{d6}/dpp^{d12} flies showed abnormalities in the distal segments. In most cases arista, the distal most antennal segment, was absent and a conical projection on the third segment presumably represented the fused fourth, fifth and the sixth antennal segments (Figure 1 *g*). As in the legs, the structural abnormalities in the antennae of B^+; dpp^{d6}/dpp^{d12} flies were further intensified in *B*; dpp^{d6}/dpp^{d12} flies (Figure 1 *h*) since the arista along with the fourth and the fifth antennal segments were completely absent while the third and the second antennal segments were widened and deformed with disruptions in the bristle pattern.

When compared with *B/B*; dpp^{d6}/dpp^{d12} female or *B/Y*; dpp^{d6}/dpp^{d12} male flies, the severity of abnormalities in legs and antennae was less in *dpp* mutant females heterozygous for *B* mutation (*B/B*$^+$; dpp^{d6}/dpp^{d12}).

The above-noted enhancing effect of *B* mutation on abnormalities in appendages due to *dpp* mutant condition, clearly suggested the possibility of Bar protein expression in the corresponding larval imaginal discs and some interaction between the *Bar* and *dpp* gene products during differentiation of these appendages from the undifferentiated discs. The leg and the antennal discs of larvae are essentially circular flattened, monolayer sac-like structures of columnar epithelial cells which evaginate during the pupal stage to give rise to the respective adult structures. The central part in both types of discs corresponds to the presumptive distal tip while the peripheral regions correspond to the progressively more proximal structures of adult appendages[12,13]. To examine if *B* genes expressed in leg and antennal discs during development, the S12 antibody, which recognizes both BarH1 and BarH2 proteins[4] was used for immunostaining the leg and eye antennal discs for *+/+*, *B*, B^+; dpp^{d6}/dpp^{d12} and *B*; dpp^{d6}/dpp^{d12} late third instar larvae. Immunostaining with the S12 antibody revealed that the BarH1 and/or BarH2 proteins were abundantly present in a group of cells arranged as a 2–4 cell wide asymmetric ring around the central part of all the leg discs of wild-type late third instar larvae (Figures 2 *a*, 3 *a*) and at two small regions in the center within the ring (as indicated by arrows in Figure 3 *a* and *b*). The ring of Bar expressing cells corresponds to the preseumptive tarsal segment as revealed by immunostaining of evaginating leg discs from 9–10 h old pupa: as seen in Figure 3 *c*, the Bar-expressing cells in these discs were essentially restricted to the developing tarsal segment. The two groups of Bar expressing cells in the center of the leg disc (arrows in Figure 3 *a* and *b*) correspond to the presumptive claws. In the wild-type antennal discs also, BarH1 and BarH2 proteins were seen in a ring around the central part of the disc (Figure 4 *b*) but without any additional sites of expression in the central region. The patterns of expression of BarH1 and BarH2 in leg and antennal discs were similar in *B* mutant larvae (Figures 2 *b*, 3 *b*) except that compared to the wild-type discs, the staining was detectably more intense (compare Figure 3 *a* and *b*). The 2–4 cell wide ring of Bar-expressing cells in wild-type as well as the *B* leg, but not the antennal discs was marked by patterned circular areas in which Bar proteins were absent (marked by asterisks in Figure 3 *a* and *b*). It is known that BarH1 and BarH2 are expressed in the thecogen and neuronal cells of embryonic external sensory organs and this plays a key role in determining the sensillum subtype[14]. Futhermore, expression of BarH1 and BarH2 in the notal region of the third instar larval wing disc regulates the formation of microchaetae[8]. Therefore, it is possible that the characteristic pattern of Bar-expressing and Bar-non-expressing cells within the ring in leg discs serves as a pre-pattern for the pattern of the microchaetae and other bristles in adult legs. This needs further detailed analysis.

Compared to the leg and antennal discs from wild-type and *B* larvae, those from B^+; dpp^{d6}/dpp^{d12} showed disruptions in the patterns of Bar expression, in agreement with the earlier noted structural abnormalities in legs and antennae of adult flies. The leg as well as the antennal disc in B^+; dpp^{d6}/dpp^{d12} larvae were somewhat deformed and the ring of *Bar*-expressing cells was distinctly smaller and displaced (see Figure 2 *c*). On closer examination it was seen that the ring of Bar-expressing cells in the leg discs lacked the typical pattern formed by the Bar-expressing and Bar-non-expressing cells in wild-type or the *B* mutant discs. This lack of the 'pre-pattern' within the Bar-expressing ring of cells perhaps correlates with the aberrant bristle and other patterns in adult legs. The two small groups of Bar-expressing cells in the center of the ring were also absent in B^+; dpp^{d6}/dpp^{d12} leg discs and this correlated with the absence of claws in adult legs of these flies.

Most interestingly, the pattern of Bar-expressing cells in the *B*; dpp^{d6}/dpp^{d12} leg discs was completely disrupted

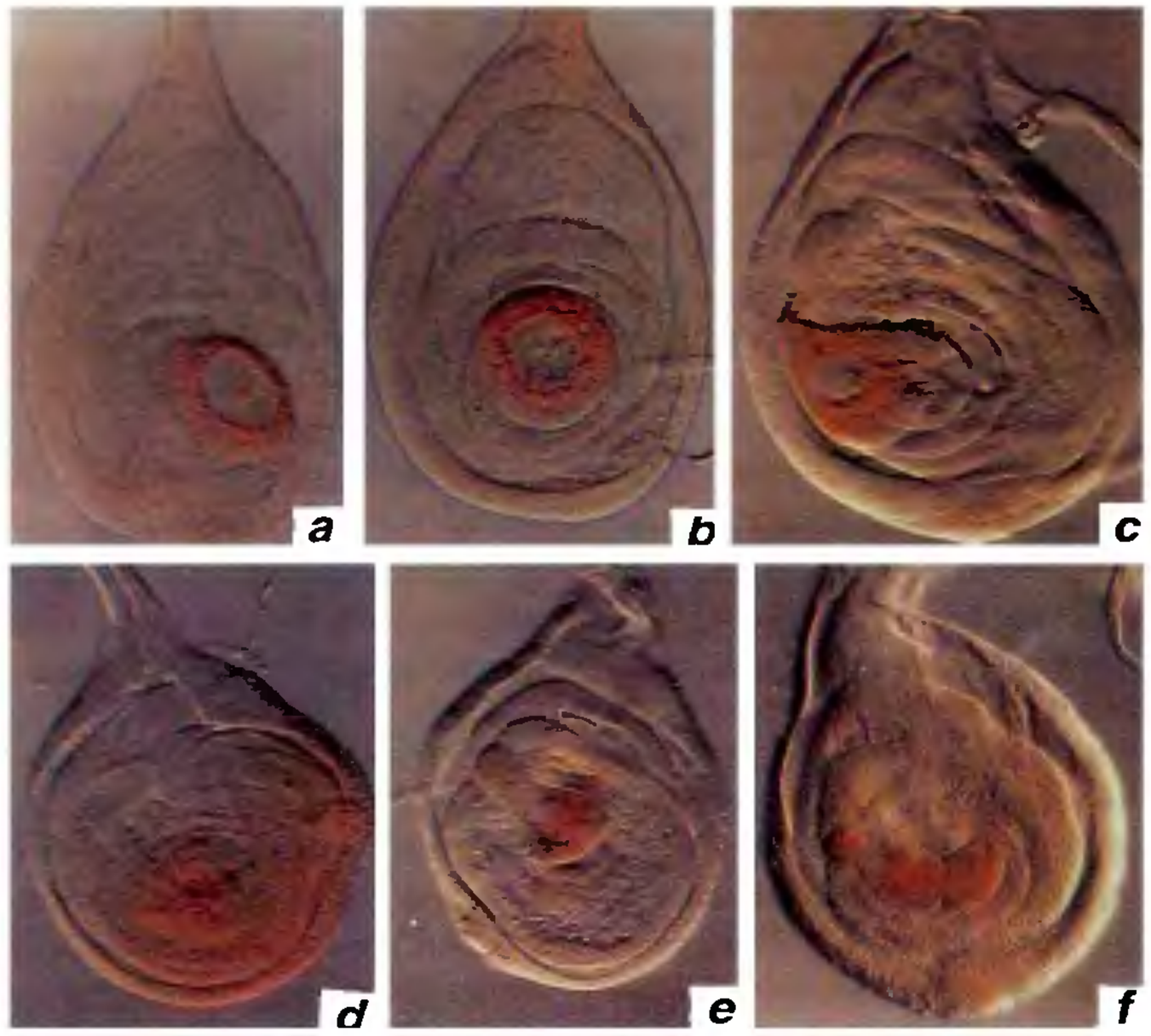

Figure 2. Expression of Bar proteins in mesothoracic leg imaginal discs of wild-type (*a*), *B* (*b*), B^+; dpp^{d6}/dpp^{d12} (*c*) and *B*; dpp^{d6}/dpp^{d12} (*d–f*) late third instar larvae as seen by immunostaining with the rabbit monoclonal antibody S12. Biotinylated anti rabbit IgG antibody and streptavidin conjugated HRP system (Vector) was used to detect the primary antibody following the standard protocol.

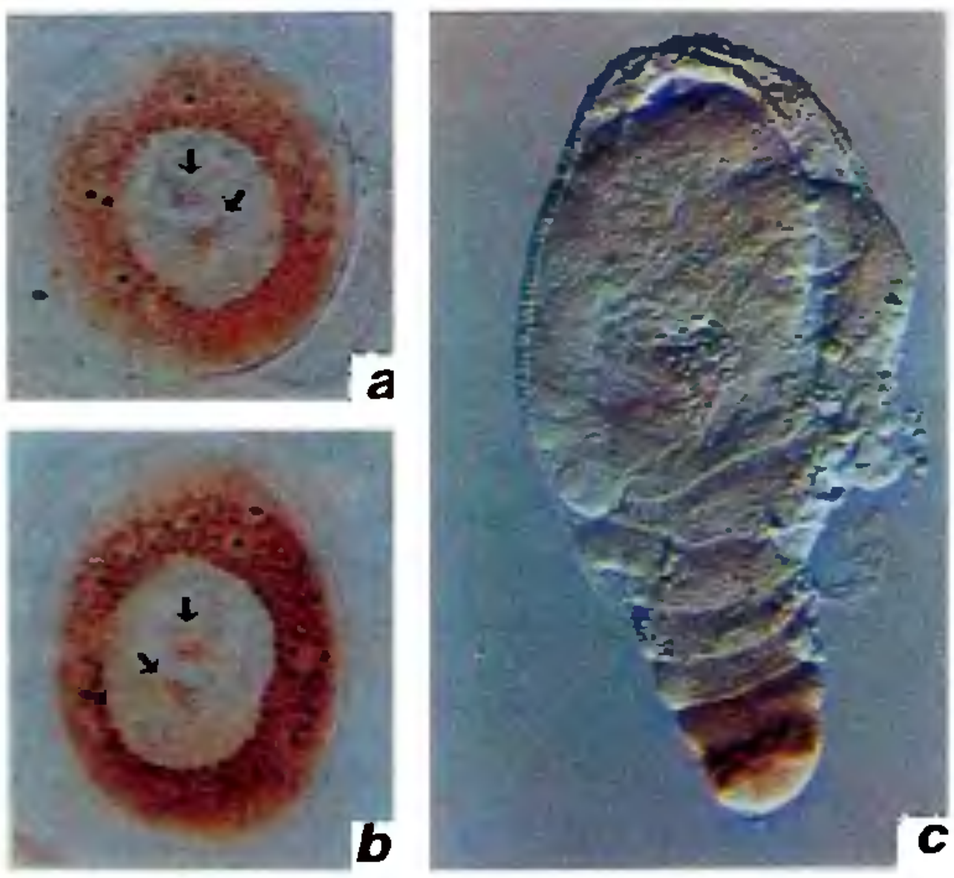

Figure 3. Enlarged view of the ring of Bar-expressing cells in presumptive tarsal region of mesothoracic leg discs of wild-type (*a*), *B* (*b*) late third-instar larvae following immunostaining as in Figure 2. The arrows in *a* and *b* point to the pair of group of Bar expressing cells in the central region of leg disc, corresponding to the distal-most segment of the adult leg. Note the more intense staining in *b* and the patterned absence (some are marked with *) of Bar expression in several areas of the ring in *a* as well as *b*. An evaginating mesothoracic leg disc from 9–10 h old pupa after immunostaining with the S12 antibody is shown in *c*.

(Figure 2 *d–f*). As evident from the examples in Figure 2 *d–f*, the ring was replaced by a group of Bar-expressing cells occupying the entire central region and additional sites of ectopic expression of Bar proteins. These cells were spread over a large area of the disc (Figure 2 *d*) or were limited to a smaller area (Figure 2 *e*, *f*). In some *B*; dpp^{d6}/dpp^{d12} leg discs, the Bar-expressing distal region (central in the disc) appeared to be duplicated (Figure 2 *f*). Change from the annular pattern of Bar-expressing cells to the uniform sheet of Bar-expressing cells in the distal region of *B*; dpp^{d6}/dpp^{d12} imaginal leg discs may perhaps be due to absence or disappearance of the distal-most group of imaginal cells since in the adult legs of these genotypes the meta-tarsal segments were almost completely absent (Figure 1 *d*). Comparable disruptions in the S12 antibody staining patterns were noted (not shown) in the antennal discs of B^+; dpp^{d6}/dpp^{d12} and *B*; dpp^{d6}/dpp^{d12} late third-instar larvae.

Since *dpp* plays a key role in proximo-distal axis differentiation and our above results showed that the *B* and *dpp* genes interact in this process, we examined the spatial relationship between the Bar-expressing and the Dpp-expressing cells in normal late third-instar leg and antennal discs. The *dpp* expression was monitored through X-gal staining in leg and eye-antennal discs of the *dpp-lacZ* transgenic line *BS3.0* (ref. 15) in which *dpp* promoter regulates the *lacZ* reporter gene. The same discs

were subsequently immunostained with the S12 antibody to localize Bar proteins (Figure 4 *a*, *b*). As is already known[16], *dpp* expression in leg and the antennal discs was restricted along the anterior–posterior boundary and accordingly, the X-gal blue staining extended as a narrow stripe along the antero-posterior axis in leg discs and as a wedge sector in antennal discs (Figure 4 *a*, *b*). In leg discs, the stripe of Dpp-expressing cells (bluish-green) crosses the ring of Bar-expressing cells (brown) at the dorsal-anterior diagonal and ends close to the ventral–anterior diagonal of the ring while in antennal disc, the overlap is restricted only to the dorsal side. (Figure 4 *a*, *b*). It may be noted that the spatial distribution of Dpp- and Bar-expressing cells in leg and antennal discs is strikingly different from that in the eye-disc where both are co-expressed all along the anteriorly advancing morphogenetic furrow and the Bar proteins, in addition, are also expressed in specific (R1 and R6) cells of the differentiating ommatidia where Dpp does not express (see Figure 4 *b*).

Growth and pattern formation in the leg and antennal imaginal discs depends mainly on cell–cell interaction rather than cell lineage[17]. The Dpp protein is a secretory protein, which in conjunction with the products of *wingless* (*wg*) and some other genes plays the most important role in specifying the positional information along the proximo–distal and dorso–ventral axes[18–20]. In addition to these, other genes have restricted expression in specific positions along the proximal–distal axis. Expression of these proximal-distal sector genes appears, when viewed from top, as annular rings in third-instar leg discs or as band/s in the everting discs and these genes provide molecular identities to different positions along the proximal–distal axis[21]. Our present study has shown that the *B* gene is also expressed in a distinct ring, corresponding to the presumptive distal region in the third-instar leg and the antennal discs. Therefore, we suggest the *B* gene to be a new member of group of proximal–distal sector genes like *distalless*[22], *teashirt*[23], *rotund*[24,25], etc. The specific band-like expression of Bar in the tarsal segments of the evaginating leg discs further supports its function as a proximal–distal sector gene in these appendages.

The dpp^{d6} and dpp^{d12} alleles are loss-of-function alleles due to deletion in the disc region of the *dpp* gene[9]. Generally such mutant alleles are recessive lethal but due to a partial complementation, the dpp^{d6}/dpp^{d12} hetero-allelic combination permits survival of a few individuals to adult stage with severe abnormalities in appendages. Aggravation of these mutant phenotypes by the *B* mutation clearly indicates that the *B* and *dpp* genes interact in leg and antennal differentiation. Although *dpp* is expressed in a stripe that roughly parallels the anterior–posterior boundary in the leg and antennal discs[16], adult viable mutants of *dpp* show abnormalities in adult appendages restricted to distal structures. This suggests that the diffusible product of this gene has a more significant role in pattern formation in the distal segments of these appendages[10,11]. Our results showed that in spite of the spatially well defined localization of Bar homeo-box-containing proteins in leg and antennal discs, their over-expression due to the *Bar* mutation in dpp^{+} background had no effect on leg or antennal differentiation but in *dpp* loss-of-function background, the *dpp*-mutant phenotypes in these appendages were significantly aggravated. This suggests some kind of stoichiometric relationship between these two gene products such that when the Dpp protein is below a certain threshold level, as is likely to happen in the dpp^{d6}/dpp^{d12} heteroallelic combination, an overdose of Bar homeo-box proteins has an enhancing effect on the *dpp* phenotype. It appears that Bar proteins have an inhibitory effect on *dpp* expression so that in the dpp^{d6}/dpp^{d12} heteroallelic combination, the already lowered activity of Dpp gets further reduced resulting in more extreme phenotypes. The disruption in the pattern of Bar expression in leg and antennal discs in *dpp* mutant background (Figure 2 *c*) suggests a role of Dpp in regulating *B* expression: While in the eye-disc, the *B* and *dpp* express in spatially overlapping regions (see Figure 4 *b*), in the case of leg and antennal discs (Figure 4), there is only a limited overlap between the expression domains of these two genes. In spite of this limited overlap, the Bar-expressing annulus in *dpp* mutant discs was reduced and in the double mutants, it was completely disorganized. These suggest either a direct long-range field effect of Dpp on *B* expression or an indirect effect resulting from the altered expression of *wg*, *hedgehog* (*hh*) and other

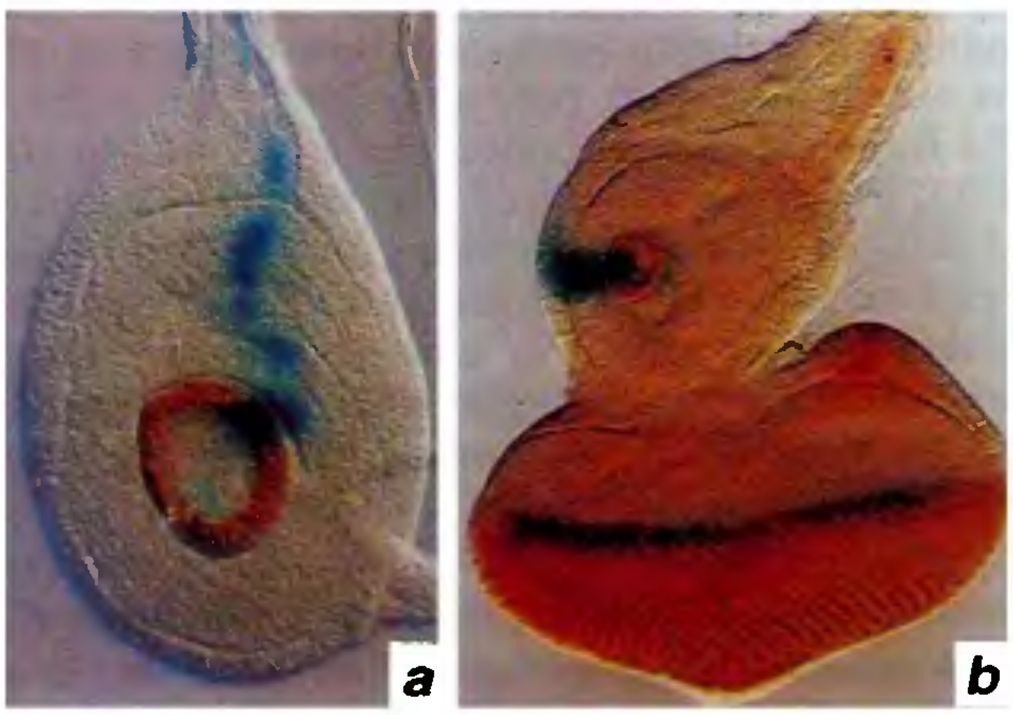

Figure 4. Mesothoracic leg (*a*) and eye-antennal (*b*) imaginal discs from the transgenic line *BS3.0* stained with X-gal to reveal the *dpp* expression pattern (bluish-green) followed by immunostaining with the S12 antibody to show the Bar expression (brown) pattern. In *b*, the lower half is the eye-disc and the upper half, the antennal disc. Note that the *dpp* expression in the leg and antennal discs is along the antero-posterior border while in the eye-disc it is restricted to the morphogenetic furrow (MF). In eye disc, Bar is expressed in the MF as well as in specific cells within each of the differentiating ommatidia arrayed posterior to the MF.

genes due to *dpp* loss-of-function mutation. This aspect is being examined further.

As has been reported earlier[26], most of the B^+; dpp^{d6}/dpp^{d12} males were completely devoid of external genitalia while a few had abnormal external genitalia. As in legs and antenna, the *Bar* mutation had an enhancing effect on male genitalia also since the external genitalia were absent in all *B*; dpp^{d6}/dpp^{d12} male flies (not shown). Interestingly, the external genitalia were not much affected in female flies of any of the genotypes. The enhancing effect of *B* mutation on male, but not female, external genitalia in *dpp* mutant background also warrants further study.

The classical view has been that the *Bar* locus has a function only in eye differentiation in view of its phenotypic effect being restricted to differentiation of ommatidia in eyes. Recently, this gene was shown to also function in the differentiation of the notal region of wing of *Drosophila*[8]. We have now shown that the *Bar* locus has roles in differentiation of legs and antennae (and possibly also the external male genitalia) as well. Thus it appears that this complex locus of homeo-box containing genes plays a much wider role in differentiation of different structures in *Drosophila*.

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Isolation and characterization of PR1 homolog from the genomic DNA of sandalwood (*Santalum album* L.)

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Genomic library was constructed using nuclear DNA prepared from tender leaves of sandalwood. Subsequently, screening with heterologous probes we could isolate the PR1 genomic homolog. Restriction mapping and hybridization experiments were carried out to obtain the coding region for *PR1* gene. A 750 bp *Eco*RI fragment thus obtained was subcloned to yield pSaPR1, which was compared with the related sequences. Southern hybridization with genomic DNA digests was carried out to check its genomic organization. The induction of this gene was observed in the somatic embryos treated with salicylic acid, thereby implying its possible involvement during systemic acquired resistance.

SELF defense in plants stems from the necessity of their survival against various pathogens. As observed, plants are being challenged constantly by various pathogens but disease is not always the inevitable outcome. Depending on the pathogen, plants exhibit different types of defense responses which can be classified into three classes according to their spatial and temporal occurrences[1]. The first class comprises immediate, early responses that

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involve changes in ion fluxes across the plasma membrane, synthesis of active oxygen species (oxidative burst)[2–4] and the hypersensitive reaction (HR). HR is characterized by a local necrotic lesion that effectively traps the pathogen to the site of infection and prevents its spread throughout the rest of the plant[4]. The second line of defense, thought to restrict the growth and development of pathogen, is activated at the site of infection. This response involves the *de novo* synthesis of several proteins including enzymes involved in phenylpropanoid metabolism, and the biosynthesis of phytoalexins and pathogenesis-related (PR) proteins. The third line of defense that can occur in many plant–pathogen interactions is triggered on in the non-infected parts of the plant, which is known as systemic acquired resistance (SAR). SAR is characterized by the protection of uninfected parts of a plant against a second infection by the same or even unrelated pathogen[5,6]. SAR implies the existence of a signal molecule produced in the infected tissue, that moves throughout the plant to activate resistance[7]. Salicylic acid (SA) has been proposed to have a central role as a signaling molecule leading to SAR as its concentration rises dramatically after pathogen infection[8–13]. Furthermore, exogenously applied SA leads to typical SAR responses such as increased resistance to viral infection[5,14,15]. Recent evidence suggests that SA may not be the primary long-distance SAR inducing signal and that the production of this systemic signal is not dependent on SA accumulation. However, SA is required in uninfected tissues for transduction of the translocated signal into gene expression and resistance[16].

SAR is associated with the systemic *de novo* synthesis of a large number of PR proteins. Their time of appearance and the known function of at least some of the PR proteins suggest their involvement in SAR. Some members of the PR family, chitinases and β-1,3-glucanases, inhibit fungal growth. Moreover, β-1,3-glucanases may release defense-activating elicitors. Direct evidence of the potential role of PR genes in plant defense has been obtained by the experiments which demonstrated that overexpressing PR genes can lead to enhanced resistance to certain pathogens[17,18]. Since PR genes are induced in parallel with the appearance of SAR, they are useful targets to develop protection strategies in plants. Moreover, in the systems where genetically defined resistance is not described or prior knowledge of pathogen avirulence gene is not available, development of disease resistance may be achieved by manipulating these sets of genes.

As part of a tree improvement programme we are trying to study the defense response in an economically important tropical timber tree sandalwood (*Santalum album* L.), which may be used later to develop disease-resistant plants. Towards this end, we have attempted to clone and characterize some of the PR genes from sandalwood. Here we report cloning and characterization of PR1 homolog from sandalwood genomic library. We could demonstrate the induction of this gene in the somatic embryos when treated with salicylic acid, thereby implying its possible induction during SAR.

Somatic embryos used for this study were obtained by direct somatic embryogenesis (G. Lakshmi Sita *et al.* unpublished work) from internodal segments of young shoots. Briefly, explants were inoculated in MS medium[19] supplemented with thidiazuron (TDZ) and 6-benzylaminopurine (BAP) for direct somatic embryogenesis. Globular embryos thus obtained were transferred to MS medium supplemented with gibberellic acid (GA). Three- to 4-week-old somatic embryos were used for induction with SA and other downstream applications.

High molecular weight DNA was prepared from the nuclei isolated from sandalwood leaves. Briefly, sandalwood leaves were frozen and pulverized in liquid nitrogen which was then suspended in 5 volumes of cold nuclei isolation buffer (NIB) (15% sucrose, 50 mM Tris pH 8.0, 50 mM EDTA, 5 mM $MgCl_2$, 5 mM mercaptoethanol, 150 mM NaCl). Once thoroughly mixed, the homogenate was allowed to pass through three layers of cheese cloth and then centrifuged at 1500 *g* for 10 min at 4°C. The precipitate thus obtained was resuspended in the same buffer containing 0.1% Triton X-100. After an incubation in ice for 10 min, a nuclear pellet was obtained by centrifugation at 100 *g* for 10 min at 4°C. This nuclear pellet was used for isolation of high molecular weight DNA by the standard procedure[20].

DNA partially digested with *Sau*3AI was partially filled in and subsequently cloned in the λGEM-11 half-filled arms as described in Promega Protocols. The genomic library containing 105 recombinants was screened with *Arabidopsis* PR1, PR2 and PR5 cDNA probes (kindly provided by Dr John Ryals, Navartis, USA). Out of 19 positive clones obtained after multiple rounds of screening, one that was positive for PR1 was taken up for further characterization. This genomic clone having the insert of size approximately 18 kb was subjected to restriction mapping followed by Southern hybridization to obtain the coding region for PR1. A 750 bp *Eco*RI fragment thus obtained was subcloned in pBluescript to yield pSaPR1. Sequencing was carried out using Sequenase Kit (ver 2.0) (USB Biochemicals, USA) following the manufacturer's instruction. The nucleotide sequence and the deduced amino acid sequence from the same is represented in Figure 1.

Genomic DNA was restricted with *Hin*dIII, *Eco*RI, *Sac*I and *Xho*I which do not have an internal site within SaPR1 and fractionated in 0.8% agarose gels. DNA was transferred to nylon membrane (Hybond N$^+$, Amersham Inc.) using TE-80 vacuum transfer system (Hoefer Scientific Instruments, USA). Blots were hybridized with SaPR1 probe. Probes were labelled with [α-^{32}P]dATP using Amersham's megaprime labelling kit. Hybridizations were carried out for 24 h at 42°C in 50%

formamide, 5 × Denhardt's solutions, 6 × SSC, 1% SDS, 100 g ml denatured Salmon sperm DNA and blots were washed finally at 0.1 × SSC, 0.1% SDS for 30 min at 65°C.

Total RNA was isolated from sandalwood somatic embryos (treated with SA or mock treated with water) by the GITC-acid phenol extraction method as described by Chomczynski and Sacchi[21]. 10 µg of total RNA was denatured in formamide, separated by electrophoresis through formaldehyde agarose gels and blotted to Hybond N^+ filters[22]. Blots were hybridized with SaPR1 probe. Probes were labelled with [α-^{32}P]dATP using Amersham's megaprime labelling kit. Hybridizations were carried out in the same conditions as described earlier. Filters were washed finally at 0.5 × SSC, 0.1% SDS for 15 min at 50°C. All RNA gels were routinely visualized with ethidium bromide staining and equal loading was confirmed by reprobing the same blot with RNA gene probe.

The sequence of the predicted amino acids was aligned with related sequences obtained from SWISS-PROT data base. When compared with the known PR1 sequences it reveals 37–49% homology in the coding region (Table 1).

gaattcatgccatgcacttccctttactccaattggctttctctaataggggaaaagccc
tccatttccgtcaaatgacccggcctcccctggctcttccaccgtccgggcttccttcc
tccctatgctatgctcccccggcgcaggcctaccacgcttcgcgcctgcggcgttttcgc
cagacggtctttgccagtagtgccatcctccccgctggctgtatgggcgggttttccctt
M G G F S L
gcttcagcgttcttgttaagcctatggataactggaacaattgttagcagtagagctcaa
A S A F L L S L W I T G T I V S S R A Q
aatagcgcacaagattattcaacggttcccaacgtgagggcagtgggtgtagagataacc
N S A Q D Y S T V P N V R A V G V E I T
ccgtgggatgagcagcgccttgctgcatccgctcggcaacgcgcatcagatttaaaaaca
P W D E Q R L A A S A R Q R A S D L K T
cggtgccggctcgtacactctcattcgccttacggggaaaacttagccataactagcggt
R C R L V H S H S P Y G E N L A I T S G
cactttactacctttctcgccttcctccccatgtgggttgtcgagaagtttaactacgcc
H F T T F L A F L P M W V V E K F N Y A
gccacgctttgcgtttgccaccaagccgccttacggggttgcgctagaaagtcaaatggg
A T L C V C H Q A A L R G C A R K S N G
ggaaccaagcaggccagcagcaatggaccgactggagtggttccgtactagcgactcggc
G T K Q A S S N G P T G V V P Y *

gtcagcggcttcgtgccgcactggagtgatccaatatgtggttccgcacgttccaccacgaattc

Figure 1. Nucleotide and deduced amino acid sequences of the SaPR1. The genomic fragment contains 725 nucleotides. The stop codon is marked with an asterisk.

Table 1. Percentage of identity of aligned amino acid sequences of PR1

	Tobacco PR1c	Tobacco PR1a	Tobacco PR1b	*Arabidopsis* PR1	Maize PR1	
SaPR1	47.9	47.1	46.3	45.4	36.7	Per cent identity
	121	121	121	119	109	amino acid overlap

Organization of SaPR1 sequences in the genome was made by genomic blotting experiments. Genomic DNA was cut with *Eco*RI, *Hin*dIII, *Xho*I, *Sac*I restriction enzymes which do not cut within the 750 bp EcoRI fragment used as probe. As shown in Figure 2, the probe hybridizes predominantly to single fragments at high stringency in all four digests. In addition to this, other weak bands could be detected in some of the digests. To determine whether these fragments were due to partial digestion of DNA, the same digests of DNA were probed with other control DNA and the banding patterns indicated complete digestion (data not shown). Together, these results suggest that SaPR1 may react with other members of this gene family.

Expression of PR1 was checked in the somatic embryos mock treated with water and upon treatment with SA. Figure 3 shows that there is an induction of PR1, when the embryos were treated with SA. The probe hybridizes specifically to a single mRNA species of size 0.8 kb. Longer exposure of the blot results in the appearance of faint signals in the uninduced lanes (data not shown). This corroborates well with the other reports of PR1 induction in various plants, thus suggesting the possible involvement of SaPR1 during SAR.

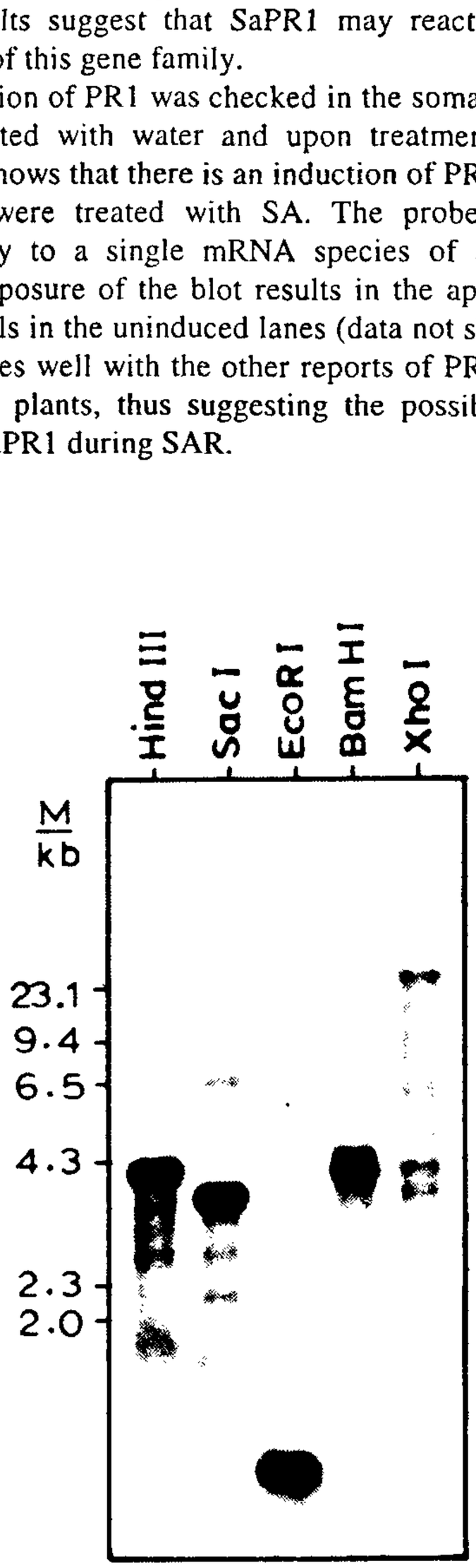

Figure 2. Southern hybridization analysis. Genomic DNA (10 µg per lane) was digested with the indicated restriction enzymes. The migration of the molecular weight standards are indicated.

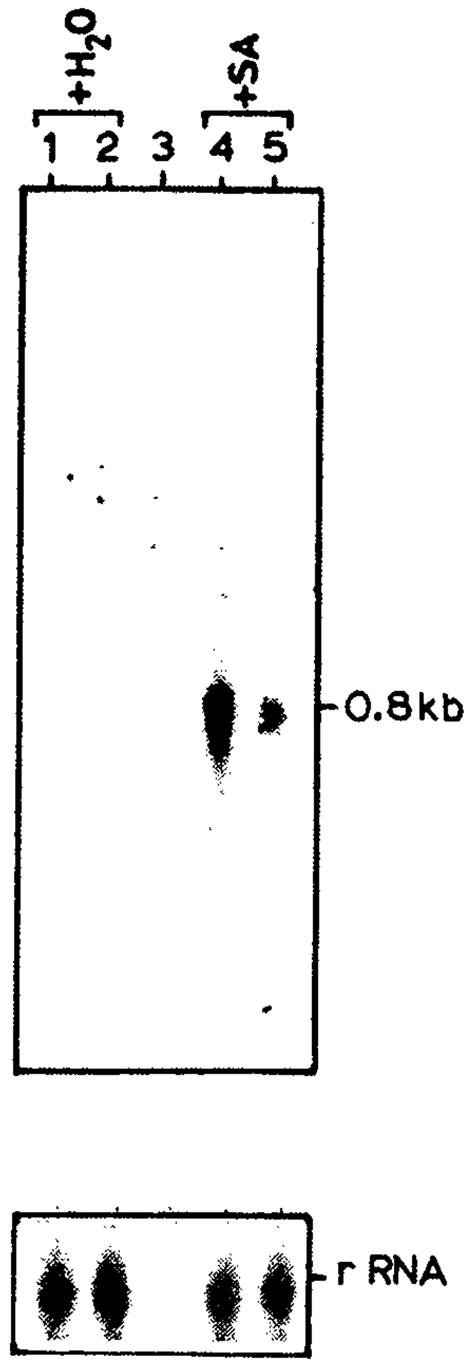

Figure 3. Expression of *SaPR1* transcript in SA-treated somatic embryos. 10 µg of total RNA on each lane from uninduced and induced embryos was loaded as indicated. Upper panel shows the expression of *SaPR1* transcript in the induced embryos. The same blot was reprobed with rRNA gene as shown in the bottom panel. Lanes 1 and 2, RNA from water-treated embryos in different periods; Lane 3, RNA from control (no treatment) embryos; Lanes 4 and 5, RNA from SA-treated embryos in different periods.

In summary, a genomic library was constructed from an economically important tropical timber tree, sandalwood and screened with *Arabidopsis* PR1, PR2 and PR5 cDNA probes. PR1 reading frame was identified from a genomic fragment, which codes for a putative protein of 143 amino acids and an estimated molecular mass of 15 kDa. The *SAR* gene shares 43–47% amino acid sequence identity with various *PR1* genes. Southern hybridization was carried out using SaPR1 probe to show its possible homology with other members of the gene family. Thus we have successfully demonstrated that this gene is induced by SA, and hence probably during SAR, by northern blot analysis.

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