Theor Appl Genet (1983) 65 : 1–8

Genetics of the peroxidase isoenzymes in *Petunia*

Part 5. Differential temporal expression of *prxA* alleles

B. M. van den Berg, F. Bianchi and H. J. W. Wijsman
Institute of Genetics, University of Amsterdam, Kruislaan 318, NL-1098 SM Amsterdam, The Netherlands

Received December 29, 1982
Communicated by H. F. Linskens

Summary. In *P. hybrida* and the putative progenitor species *P. axillaris s. l.* and *P. integrifolia s. l.* five mobility alleles of the structural gene *prxA* were found. The five alleles show differential expression during development of tissue and plant, caused by internal site mutations. Analysis of young but not yet flowering plants heterozygous for *prxA* showed that the allele *prxA3* is expressed first, followed by the alleles *prxA2, prxA5, prxA4* and *prxA1*, in that order. In mature leaves of young flowering plants the *prxA2* allele has the highest expression level, followed by the alleles *prxA3, prxA5, prxA4* and *prxA1*. In mature and old leaves of old plants the expression level of the alleles *prxA2, prxA3, prxA4* and *prxA5* is about equal, whereas that of the *prxA1* allele is about twice as high. Crossing experiments suggested that between the plants used no external site differences exist that cause clearly detectable changes in developmental allozyme balance. Fast moving anodic peroxidases were detected that have a variable mobility over a considerable distance. The probability that these enzymes are precursors of the PRXa enzymes is discussed.

Key words: *Petunia* – Peroxidase – Differential allelic expression – Internal site mutations

Introduction

The peroxidase system of *Petunia* (*Petunia hybrida*, as well as related *Petunia* species) can serve as a model system for studying the regulation of gene expression in higher plants. Three of the peroxidase structural genes, namely *prxA, prxB* and *prxC*, have been mapped to different chromosomes (van den Berg and Wijsman 1981, 1982a, b; van den Berg et al. 1982). Furthermore, the existence of several regulatory mutations that affect the temporal expression of the three peroxidase structural genes have been reported (van den Berg and Wijsman 1982a, b; van den Berg et al. 1982). Among mutations that alter the expression of structural genes, those that influence the temporal or spatial expression are particularly interesting (Paigen 1979, 1980; Scandalios 1979).

We have started our study with chromosomal location of the peroxidase structural genes, so that it may be possible to determine whether regulatory mutations that affect peroxidase activity are trans-acting external site mutations or cis-acting internal site mutations. At the moment several markers are known for each of the seven chromosomes, which makes it possible to locate external site mutations. In this paper we describe differential expression of five *prxA* alleles as recognized on the basis of electrophoretic mobility. We present results that indicate the presence of internal site mutations that are responsible for the differential developmental expression of the five *prxA* alleles.

Materials and methods

Plant material

The following inbred lines were used: *P. axillaris ssp. axillaris* S1 and S2, and *P. integrifolia ssp. inflata* S6 and S9. The origin of these lines has been given by van den Berg and Wijsman (1982a); *P. hybrida* inbred lines R51, V23, W4 and M1 (derived from the cultivars 'Royal Ruby', 'Blauzwerg', 'Pendula Cyanea' and 'Rose of Heaven', respectively; primary trisomic III with genotype *prxA1/A2/A3* derived from the progeny of (trisomic III × V23) × S9; *P. integrifolia ssp. integrifolia* D579-1, D660-1 and D660-2, and *P. integrifolia ssp. inflata* D582-1 (these four plants originated from seeds collected in South-America, see van den Berg and Wijsman 1982b).

Electrophoretic analysis and zymogram scanning

Sample preparation, electrophoretic separation of the peroxidase isoenzymes using gel system I and II, and staining for peroxidase activity were carried out as described previously (van den Berg and Wijsman 1981, 1982b; van den Berg et al. 1982). Starch gels were scanned as described by van den Berg and Wijsman (1982a).

Nomenclature of the peroxidase genes and enzymes

The notation for the peroxidase structural genes and enzymes has been given earlier (van den Berg and Wijsman 1982a, b). The definition of internal and external site mutations as given in the same papers is followed here.

Results

Differential expression of the alleles prxA1, prxA2 and prxA3

As described previously the different temporal expression of the *prxA2* allele from the inbred lines V23 (*P. hybrida*) and S6 (*P. integrifolia ssp. inflata*), when compared to the expression of the standard allele *prxA1* from the tester line R51 (*P. hybrida*), is determined by an internal site mutation (van den Berg and Wijsman 1982b).

To investigate the tissue specificity of the PRXa1a2 allozyme balance young flowering plants of the F1 (V23×R51) and the F1 (S6×R51) were analysed. As shown in Figs. 1 and 2 the ratio of PRXa1.1 to PRXa2.1 activity is dependent on the age of the tissue. In very young tissue the PRXa1.1 enzyme can hardly be detected or is absent, whereas the PRXa2.1 enzyme can easily be detected. From young to old tissue the difference in activity of the allozymes gradually gets smaller. This is not only the case for leaf and stem tissue but also for flower tissue. The PRXa allozyme balance is also dependent on the age of the plant. This can be illustrated by the fact that the older the plants, the smaller is the difference in activity of the PRXa allozymes in mature corolla tubes taken from the top of the plant. In old leaves from plants that have flowered for several months the activity of the PRXa1.1 enzyme is about twice as high as the activity of the PRXa2.1 enzyme. This systemic difference persits during further aging of the plant.

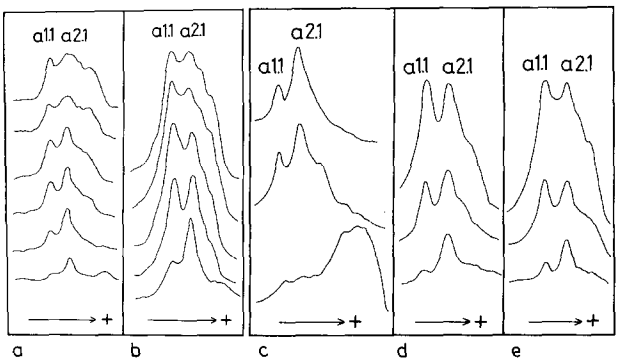

Fig. 1 a–e. Densitometric scannings of starch gels showing the PRXa allozyme balance in different parts of a young flowering F1 (V23×R51). Only symbols for the primary bands of the PRXa complex are given. **a** *From bottom to top:* young to old leaves. **b** *From bottom to top:* young to old main stem tissue. **c** Tissue taken from a flower at the first internode: *bottom:* 1.5 cm flower corolla bud; middle: calyx slip; *top:* 0.5 cm pedicel. **d** Tissue taken from a flower at the second internode: *bottom:* 3 cm tube of an opening flower corolla; middle: calyx slip; *top:* 3.5 cm pedicel. **e** Tissue taken from a flower at the third internode: *bottom: 4 cm tube of a mature flower corolla; middle: calyx slip; top:* 4 cm pedicel

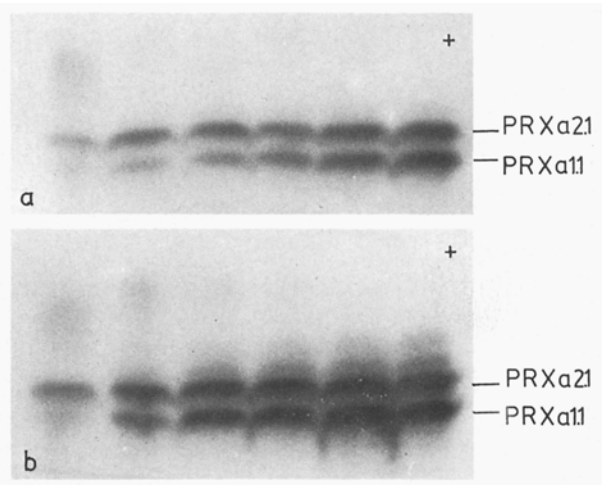

Fig. 2 a, b. Starch gels showing the PRXa allozyme balance in leaf tissue of a F1 (R51×S6) plant. From left to right, young to old leaves. **a** Analysis of a F1 plant before flowering. **b** Analysis of a young flowering F1 plant

The *prxA3* allele from the *P. integrifolia ssp. inflata* lines S6 and S9 shows, with regard to the prxA1 allele, a similar difference in temporal expression as the prxA2 allele. Among several progenies that showed segregation for the alleles *prxA1* and *prxA3*, all *prxA1/A3* heterozygotes showed the *prxA1* and *prxA3* temporal programme characteristic for the alleles of the F1 (S6×S9). Therefore, we conclude that in addition to the different temporal programme of the *prxA2* allele, with regard to *prxA1*, the different temporal programme of the *prxA3* allele is also caused by an internal site mutation.

Peroxidase enzymes with variable mobility

Analysis of young leaves from young not yet flowering plants, and flower corolla buds and tubes from young plants showed the presence of peroxidases that have a higher mobility than the PRXa bands. A striking feature of these bands is their variable electrophoretic mobility. Figure 3 shows that the mobility of the variable

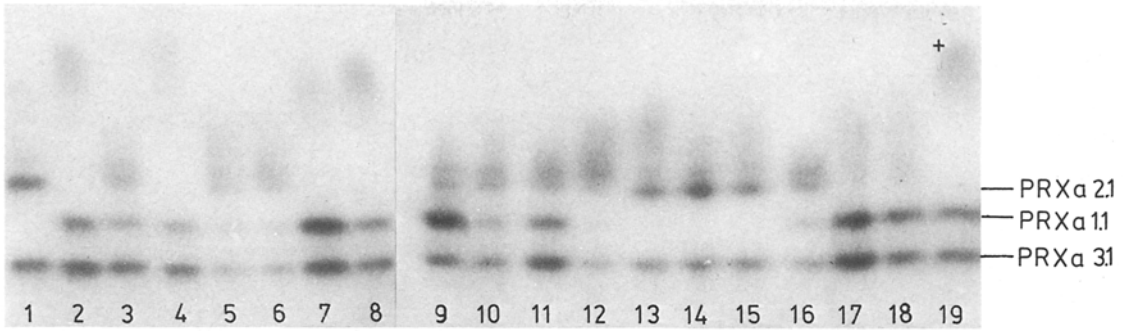

Fig. 3. Starch gel showing segregation for *prxA*, and the presence of the variable peroxidases. Leaves were taken from plants of the progeny of (W4×S6)×S6 with genotype *prxA1/A2*×*prxA3/A3*. Only symbols for the primary bands of the PRXa complex are given. Nrs. 2–12 and 16–19 show *prxA1/A3* heterozygotes, and nrs. 1 and 13–15 *prxA2/A3* heterozygotes. Nrs. 2, 4, 7, 8 and 19 show variable bands more near the anode. Nrs. 3, 5, 6, 9–12 and 16 show two variable bands around the position of the PRXa2.1 enzyme

bands ranges over a considerable distance. Only at a lower mobility two bands can be seen. This was only the case for plants heterozygous but not for plants homozygous for *prxA*.

The presence of the variable bands is highly dependent on the age of the tissue and plant. Their presence is characteristic for young tissue of young plants. In leaves of young plants they can easily be detected, but barely in young and never in mature leaves of old plants. In flower corolla tissue they can mostly be detected, especially in flower corolla buds, regardless the age of the plant. Analysis of leaves from a young not yet flowering plant showed that the older the tissue, the more anodal proximate is the position of the variable bands (Fig. 6).

In gel system II the variable bands can also be detected (Fig. 4). However, both for *prxA* homozygous and heterozygous plants always only one sharp variable band was detected. The mobility of the variable band with a higher mobility than the PRXa 1.1 enzyme decreases during development of the flower. The acivity of the variable peroxidase decreases during aging. This decrease goes hand in hand with the increase in activity of the PRXa 1.1 enzyme.

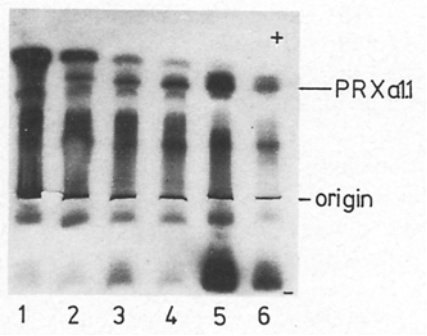

Fig. 4. System II starch gel showing peroxidase isoenzymes present in flower corolla tissue of different age from line M1. Nrs. 1–4, flower corolla buds (young to older). Nr. 5, 6, flower corolla tube and limb, respectively, of a mature flower corolla. The older the flower corolla bud the lower is the mobility of the variable band (the anodic band with the highest mobility)

These observations are important in view of the PRXa allozyme balance studies. First, the activity of the variable bands may interfere with properly scoring the allozyme balance in young tissue. Second, the variable bands may be precursors of the PRXa enzymes (see Discussion).

The prxA2 and prxA3 internal site mutations

As was shown above, the alleles *prxA2* and *prxA3* differ from the allele *prxA1* in temporal expression, the *prxA1* allele having a delayed temporal programme caused by internal site differences. Analysis of plants with genotype *prxA2/A3* showed a more complex difference in temporal expression between both *prxA* alleles.

In young tissue the presence of the variable peroxidases complicated the analysis of the PRXa allozyme balance of *prxA2/A3* plants. Figure 5 shows that the variable peroxidases contribute to the peak height of the PRXa 2.1 band, which becomes therefore higher than the one of the PRXa 3.1 band. When the variable bands do not interfere, the PRXa 3.1 peak height is higher. In fact, in flower corolla buds of young plants the PRXa 2.1 enzyme may be undetectable, whereas the PRXa 3.1 enzyme is present. The activity of the PRXa 2 bands increases relatively during further development of leaf tissue. In mature leaves of flowering plants every PRXa 2 enzyme has a higher activity than its corresponding allozyme (Fig. 3, van den Berg and Wijsman 1982 b). In old leaves of flowering plants the allozymes have a similar activity.

The difference between the temporal programming of the *prxA2* and *prxA3* alleles has been found in all *prxA2/A3* heterozygotes from backcross and F 2 progenies consisting of more than 300 plants.

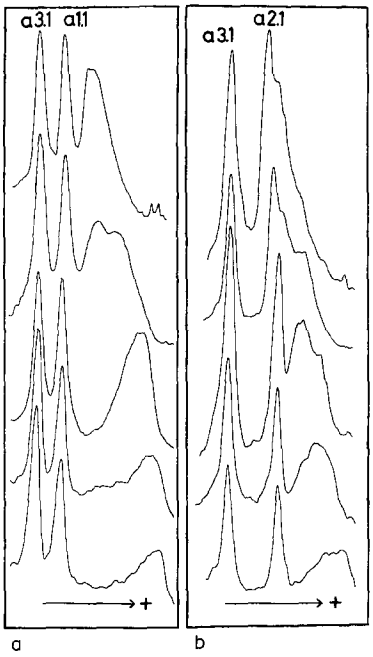

Fig. 5 a, b. Densitometric scannings of starch gels showing differential expression of the alleles *prxA1, prxA2* and *prxA3* in the progeny of the B1 (W4×S6)×S6, and the electrophoretic variation of the variable bands. Samples were young and mature leaves of young not yet flowering plants. The anode is at the right of each scanning. Only symbols for the primary bands of the PRXa complex are given. **a** Analysis of plants with genotype *prxA1/A3*. The second tracing from the top shows 2 variable bands. **b** Analysis of plants with genotype *prxA2/A3*. It can be seen that the lower the mobility of the variable peroxidases the higher is the height of the PRXa2.1 peak relative to the PRXa3.1 peak height

We conclude that the difference in temporal programming is caused by internal site difference. This holds for the *prxA2* allele from the lines V 23 and S 6, and the *prxA3* allele from the lines S 6 and S 9. We conclude that the differential expression of the *prxA2* and *prxA3* alleles with regard to that of the *prxA1* allele is caused by two different internal site mutations, and that no external site mutations are involved, that cause a detectable change in allozyme balance (Fig. 9).

The prxA4 internal site mutation

A plant obtained from seeds collected in South-America, and labelled D 582-1 (*P. integrifolia ssp. inflata*), appeared to have the genotype *prxA2/A4*. The PRXa 4.1 enzyme has a slightly lower electrophoretic mobility than the PRXa 2.1 enzyme (Fig. 6).

In young tissue the PRXa 2.1 enzyme has a higher activity than the PRXa 4.1 enzyme. In older tissue the allozyme balance cannot be determined properly due to overlap of all PRXa bands.

D 582-1 was crossed to *P. axillaris ssp. axillaris* S 2 (*prxA1/A1*). Analysis of the progeny showed that the

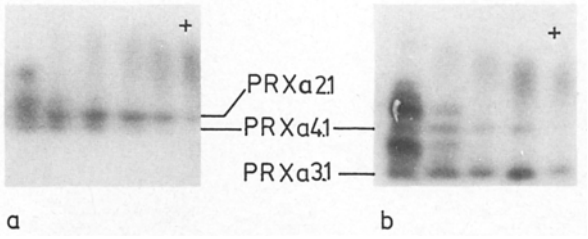

Fig. 6 a, b. Starch gels showing the differential temporal expression of the alleles *prxA2, prxA3* and *prxA4*. **a** Analysis of young to yold leaves of a young plant genotype *prxA2/A4* from the progeny of D579-1×D582-1. **b** Analysis of a young plant with genotype *prxA3/A4* from the progeny of trisomic III×(D579-1×D582-1). Note that the variable bands of older leaves are more anode proximate

PRXa 4.1 enzyme has a higher activity than the PRXa 1.1 enzyme but in older tissue a lower activity (Table 1). In flower corolla buds the PRXa 1.1 enzyme may be absent in contrast to the PRXa 4.1 enzyme.

From the progeny of D 582-1×S 2 a plant with genotype *prxA1/A4* was isolated and crossed to the trisomic III with genotype *prxA1/A2/A3* (Table 1). Analysis of the progeny showed a lower initial expression level of the *prxA4* allele with regard to the alleles *prxA2* and *prxA3* and a higher initial expression level with regard to the allele *prxA1*. In older tissue the alleles *prxA2, prxA3* and *prxA4* reached a similar expression level, whereas the level of the *prxA1* allele was about be twice as high.

From the progeny of D 579-1×D 582-1 (*prxA2/A2×prxA2/A4*) a plant with genotype *prxA2/A4* was isolated and crossed to a trisomic III with genotype *prxA1/A3/A5* (see next paragraph for the origin of this trisomic). Among the progeny all six expected types were found (Table 1). The analysis of allozyme balance of leaves of the young plants during the onset of flowering showed that the *prxA2* allele has the highest expression level, followed by the alleles *prxA3, prxA5* and *prxA4*, whereas the *prxA1* allele has the lowest expression level (Fig. 7). Again, in older tissue the alleles *prxA2, prxA3, prxA4* and *prxA5* reached a similar expression level, whereas the activity of the PRXa 1 complex was about twice as high as the other PRXa complexes.

We conclude from these results that the temporal expression of the allele *prxA4* is different from that of the alleles *prxA1, prxA2, prxA3* and *prxA5*, and that its expression is determined by an internal site mutation (Fig. 9).

The prxA5 internal site mutation

The allele *prxA5* was found in plants obtained from seeds of *P. integrifolia ssp. integrifolia* from Brazil. To obtain information about differential expression of the *prxA5* allele with regard to the alleles *prxA1, prxA2*

Table 1. Summary of essential crosses. For origin of the parents see text and the materials and methods' section

Cross and genotypes	Genotypes and numbers of diploid progeny
1. D582-1×S2	*A1/A2* *A1/A4*
prxA2/A4×prxA1/A1	7 11
2. tris III×(D582-1×S2)	*A1/A4* *A1/A1* *A1/A3* *A1/A2* *A2/A4* *A3/A4*
prxA1/A2/A3×prxA1/A4	1 0 1 1 12 11
3. D579-1×D582-1	*A2/A2* *A2/A4*
prxA2/A2×prxA2/A4	20 8
4. tris III×(D579-1×D582-1)	*A1/A2* *A1/A4* *A2/A3* *A3/A4* *A2/A5* *A4/A5*
prxA1/A3/A5×prxA2/A4	2 1 9 8 21 10
5. D660-1×D660-2	*A2/A5* *A5/A5*
prxA2/A5×prxA5/A5	11 24
6. tris III×D660-1	*A1/A2* *A1/A5* *A2/A2* *A2/A5* *A2/A3* *A3/A5*
prxA1/A2/A3×prxA2/A5	0 3 0 6 0 5
7. tris III×(tris III×D660-1)	*A1/A3* *A1/A5* *A2/A3* *A2/A5* *A3/A3* *A3/A5*
prxA1/A2/A3×prxA3/A5	3 4 24 12 11 6
8. (tris III×D660-1)×	*A3/A3* *A3/A5* *A5/A5*
(tris III×D660-1)	30 18 0
prxA3/A5×prxA3/A5	

and *prxA3*, two plants were used, namely D660-1 and D660-2, with genotype *prxA2/A5* and *prxA5/A5*, respectively.

Plant D660-1 showed to have a *prxA5* allele with a delayed temporal programme with respect to its *prxA2* allele. In young leaves the PRXa 5.1 enzyme has a lower activity than the PRXa 2.1 enzyme, whereas in older tissue the allozymes have a similar activity. All *prxA2/A5* heterozygotes (Table 1) obtained by selfing of D660-1 and crossing of D660-1 to D660-2 showed the lower initial expression level of the *prxA5* allele compared to the *prxA2* allele.

D660-1 (*prxA2/A5*) was crossed to the trisomic III with genotype *prxA1/A2/A3*, and the resulting progeny was analysed for differential expression of *prxA* alleles during development of leaves and plants. Among the diploid progeny only three PRXa phenotypes were found, namely PRXa 1 a 5, PRXa 2 a 5 and PRXa 3 a 5; the *prxA2* allele from D660-1 was not transmitted to the progeny (Table 1). The *prxA5* allele showed a lower expression level in young tissue compared to the alleles *prxA2* and *prxA3*, but a higher compared to *prxA1*. In old leaves the alleles *prxA2*, *prxA3* and *prxA5* show a similar expression level, whereas the *prxA1* allele showed an expression level about twice as high as that of the other alleles.

Two trisomics III were found among the progeny of the cross trisomic III×D660-1 : 1 plant with genotype *prxA1/A3/A5* and 1 plant with genotype *prxA2/A3/A5*. They showed similar differential expression of the *prxA5* allele with regard to the other three alleles as in the diploid progeny.

A plant from the progeny of the cross trisomic III×D660-1 with genotype *prxA3/A5* was isolated and used for further crossing experiments. The plant was selfed, and crossed to the trisomic with genotype *prxA1/*

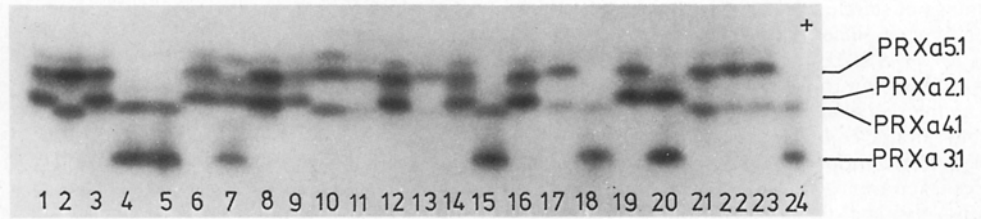

Fig. 7. Starch gel showing segregation for prxA and differential expression of *prxA* alleles in the progeny of the cross trisomic III×(D579-1×D582-1) with genotype *prxA1/A3/A5×prxA2/A4*. Young leaves of young flowering plants were analysed. Only symbols for the primary bands encoded by the five alleles are given. Nrs. 1, 3, 6, 8, 9, 12, 14, 16, 19 show the phenotype of plants with genotype *prxA2/A5;* PRXa2.1 shows a higher activity than PRXa5.1. Nrs. 2, 10, 11, 13, 17, 21–23 show the phenotype of plants with genotype *prxA4/A5;* PRXa4.1 shows a lower activity than PRXa5.1. Nrs. 4, 5, 15, 18, 24 show the phenotype of plants with genotype *prxA3/A4;* PRXa4.1 shows a lower activity than PRXa3.1. Nrs. 7, 20 show the phenotype of plants with genotype *prxA2/A3;* PRXa2.1 shows a slightly higher activity than PRXa3.1

A2/A3 (Table 1). The results again show that in the relevant plants of both progenies at the onset of flowering the allele *prxA5* has a lower initial expression level than the alleles *prxA2* and *prxA3*, and a higher than the allele *prxA1*.

We conclude that the differential expression of the alleles involved is caused by internal site mutations (Fig. 9), and that no external site mutations are involved that cause a detectable change in allozyme balance.

Differential expression of prxA alleles in flowers of trisomics III

The first flower corolla bud, just before opening of the flower corolla, of a trisomic III with genotype *prxA1/A3/A5* was analysed for developmental PRXa allozyme balance. Figure 8 a shows that in extract of flower corolla tissue only the PRXa 3.1 enzyme could be detected properly, whereas in the calyx slip all three primary bands were present. At an older age of the plant also the PRXa 5.1 enzyme could clearly be detected in an extract of flower corolla tissue, but not the PRXa 1.1 enzyme. Scan 3 of Fig. 8 a shows a variable band, in contrast to scans 2 and 4.

Analysis of a flower corolla bud of a young trisomic III with genotype *prxA1/A2/A3* showed that only the *prxA3* allele is expressed in this tissue. Figure 8 b shows that at a much older age of the trisomic also the PRXa 2.1 enzyme can be detected in that tissue; the alleles *prxA2* and *prxA3* show a similar expression level. But expression of the *prxA1* allele still can barely be detected.

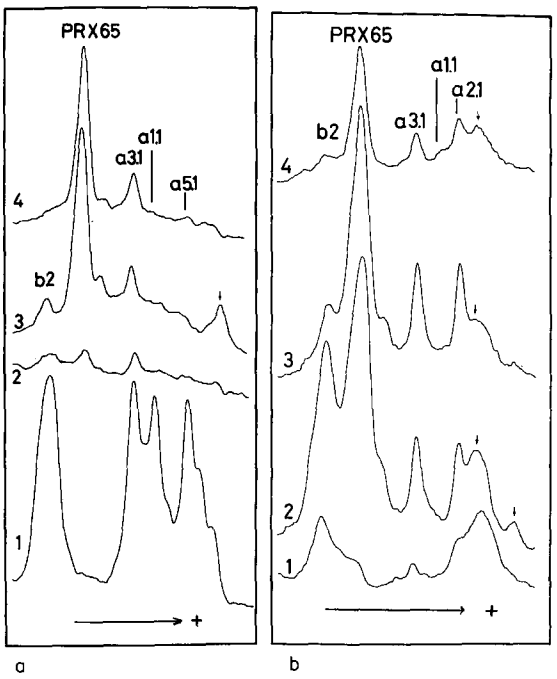

Fig. 8 a, b. Densitometric scannings of starch gels showing the differential timing of expression of *prxA* alleles in flower tissue of trisomics III with genotypes *prxA1/A3/A5* and *prxA1/A2/A3*. **a** Samples taken from a young flowering trisomic III. Nr. 1 mature leave; nrs. 2–4, *bottom, middle and top,* respectively of a 5 cm flower corolla bud. In nrs. 2–4 only the activity of the PRXa3.1 enzyme can be detected properly. Nr. 3 shows a variable peroxidase. **b** Samples taken from a trisomic III that had flowered for several months. Nrs. 1–4, tissue taken from *top to bottom* of a 5 cm flower corolla bud. Note that the expression of the *prxA1* allele cannot be detected properly, except for nr. 4. Variable peroxidases can be seen in all four scannings, nr. 2 shows 2 variable bands. The variable peroxidases are indicated with an arrow. PRX65 denotes a peroxidase that can only be detected in flower corolla tissue (van den Berg and Wijsman 1981)

Discussion

In the present paper data are presented relating to differential expression of five mobility alleles of the gene prxA. The differential temporal expression is caused by internal site mutations. Differential temporal expression of the alleles did not only manifest itself as differences in increase of enzyme acitivity, but also as difference in start of expression.

Analysis of *prxA* heterozygotes showed that the expression of the *prxA3* allele starts first, with *prxA2, prxA5, prxA4* and *prxA1* following, in that order. For the alleles *prxA2, prxA3, prxA4* and *prxA5* final expression levels are more or less equal, whereas the final expression level of the *prxA1* allele is about twice as high. Crossing experiments showed that between the plants used no external site differences exist, that cause detectable changes in developmental allozyme balance. However, we must stress the following three points.

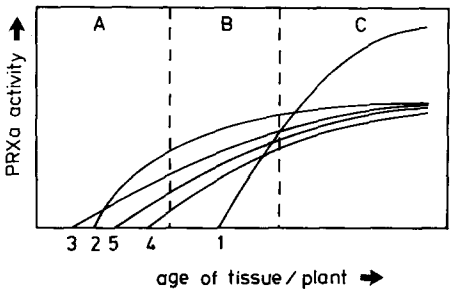

Fig. 9. Diagrammatic presentation of differential expression of *prxA* alleles (only the allele numbers are indicated). The diagram is not based on measurements of enzyme activity, but is only based on allozyme balance studies. It serves to illustrate the differences in start of expression and differences in increase of PRXa activity in tissue of different age from young and older plants. *Zone A:* flower corolla buds and tubes, and young leaves of young plants; *Zone B:* mature leaves of young plants and young leaves of older plants; *Zone C:* mature and older leaves of old plants

First, external site differences that may cause minor differences in developmental expression between two *prxA* mobility alleles remain undetectable. Measurements of peroxidase activity of a certain tissue at a defined developmental stage in order to reveal these minor changes is not feasible. (The amount of peroxidase is highly dependent on age of tissue and plant. Moreover, the activity of a single peroxidase is hard to obtain due to the high number of peroxidase isoenzymes.)

Second, external site mutations can behave dominant or recessive (Paigen 1979). Thus, any absence of the action of external site mutations can only be proved by analysis of developmental allozyme balance of large number of F 2 plants or plants obtained by backcrossing of the F 1 with both F 1 parents.

Third, the action of external site mutations in respect to PRXa developmental allozyme balance can only be detected by virtue of internal site differences between the two mobility alleles, because the alleles must be responsive to the external signal in a different extent. Without internal site differences such external signals will affect the expression of both alleles in the same way, and thus remain undetectable.

Thus, the relevance of the results presented here lie primarily in the presentation of internal site differences between the 5 mobility alleles.

The variable peroxidases can be found on a system I starch gel from the position of the PRXa 2.1 enzyme to a more anodic position (Fig. 3). This variation in position may be explained by the assumption that the variable bands are precursors of the primary PRXa bands (the bands with the lowest mobility of the variants of the PRXa complex). The additional bands of the PRXa complex, termed mozymes, are formed by post-transcriptional modification (van den Berg and Wijsman 1981, 1982 b). It may be that the formation of the mozymes is caused by interaction of the primary bands with phenolics. Van Huystee (1977) showed that interaction of one peanut peroxidase with phenolics gives rise to four additional anodic peroxidases.

Peroxidases are known to consist of a peptide and sugar moiety with a protoporphyrin 1X as prosthetic group. (Clarke and Shannon 1976; Welinder 1979; van Huystee and Cairns 1982). Variation in mobility of the variable peroxidases may reflect processing steps in the maturation of the peroxidase peptide into a glycoprotein. The order of events in the biosynthesis of peroxidases makes it possible to detect peroxidase activity of the unmaturated glycoprotein. Studies on the membrane fractions of cultured peanut cells indicated that the majority of peroxidase was synthesized on membrane bound polysomes (Stephan and van Huystee 1981). Furthermore, peptide synthesis is completed before attachment of the sugar moiety (Lew and Shannon 1973), which probably takes place in the rough endoplasmatic reticulum and Golgi apparatus (van Huystee and Cairns 1982). The precursor hypothesis implies that the unmaturated peroxidase contains the prosthetic group.

In addition to the variation in mobility and the final mobility of the variable peroxidases (the position of the PRXa 2.1 enzyme), the following observations indicate that the variable peroxidases can represent the precursors of the primary PRXa bands. In plants with genotype *prxA1/A3* and *prxA2/A3* two variable peroxidases can be found (Fig. 3), whereas plants homozygous for the gene *prxA* never showed two variable bands. In view of the precursor hypothesis triply heterozygous trisomics III are expected to contain three variable peroxidases. since three primary bands are present. Analysis of the trisomic III with genotype *prxA1/A2/A3* showed one major and one minor variable band (Fig. 8 b, scan 2). The major activity may represent coinciding precursors for two of the primary bands (observe the scans of Fig. 5), whereas the minor variable band may represent the precursor for the third primary band.

Since absolute linkage is to be expected, in a genetic test the number of primary bands and variable bands has to be the same. But the unpredictable phenotype as to variable bands of *prxA* heterozygotes made this test not feasable. Often, heterozygotes show one variable band. This may be due to poor resolution of the variable bands.

We must note here that presence of two variable bands could only be detected in gel system I and not in gel system II. In gel system II always one sharp variable band was seen for both homozygous and heterozygous plants. We can only explain this by a difference in resolution of the two gel systems.

From the genetic point of view the precursor hypothesis is interesting for the following reason. The difference in mobility of the primary bands encoded by the alleles *prxA1, prxA2* and *prxA3* may be explained by differences in processing. The variable bands are found in practically every position from a place near the anode to the position of the PRXa 2.1 enzyme. Thus the difference in position between the bands PRXa 2.1 and PRXa 1.1, and between PRXa 2.1 and PRXa 3.1 may represent differences in processing. But the mutation that caused that difference in electrophoretic mobility is located in the structural gene. Welinder (1979) showed that peroxidase from horseradish contains 8 sugar chains attached to asparagine residues. It may be possible that the difference in mobility of the primary bands is partly explained by mutations that caused replacement of an amino acid that is essential for the attachment of a carbohydrate chain.

At the moment PRXa enzymes have been purified and antibodies will be raised against them. This will enable us to test the possibility that the variable bands are reactive aginst the PRXa antibodies.

References

Berg BM van den, Wijsman HJW (1981) Genetics of the peroxidase isoenzymes in *Petunia*. Part 1: Organ specificity and general genetic aspects of the peroxidase isoenzymes. Theor Appl Genet 60:71–76

Berg BM van den, Wijsman HJW (1982a) Genetics of the peroxidase isoenzymes in *Petunia*. Part 2: Location and developmental expression of the structural gene *prxB*. Theor Appl Genet 61:297–303

Berg BM van den, Wijsman HJW (1982b): Genetics of the peroxidase isoenzymes in *Petunia*. Part 3: Location and developmental expression of the structural gene *prxA*. Theor Appl Genet 63:33–38

Berg BM van den, Hendriks T, Wijsman HJW (1982) Genetics of the peroxidase isoenzymes in *Petunia*. Part 4: Location and developmental expression of the structural gene *prxC*. Theor Appl Genet 64:75–81

Clarke J, Shannon LM (1976) The isolation and characterization of the glycopeptides from horseradish peroxidase isoenzyme C. Biochim Biophys Acta 427:428–442

Huystee RB van (1977) Relationship of the heme moiety of peroxidase and the free heme pool in cultured peanut cells. Z Pflanzenphysiol 84:427–433

Huystee RB van, Cairns WL (1982) Progress and prospects in the use of peroxidase to study cell development. Phytochemistry 21:1843–1847

Lew JY, Shannon LM (1973) Incorporation of carbohydrate residues into peroxidase isoenzymes in horseradish roots. Plant Physiol 52:462–465

Paigen K (1979) Genetic factors in developmental regulation. In: Scandalios JG (ed) Physiological genetics. Academic Press, New York, pp 2–61

Paigen K (1980) Temporal genes and other developmental regulators in mammals. In: Leighton T, Loomis WF (eds) The molecular genetics of development. Academic Press, New York, pp 419–470

Scandalios JG (1979) Control of gene expression and enzyme differentiation. In: Scandalios JG (ed) Physiological genetics. Academic Press, New York, pp 64–107

Stephan D, Huystee RB van (1981) Some aspects of peroxidase synthesis by cultured peanut cells. Z Pflanzenphysiol 101:313–321

Welinder KG (1979) Amino acid sequence studies of horseradish peroxidase. Amino and carboxyl termini, cyanogen bromide and tryptic fragments, the complete sequence, and some structural characteristics of horseradish peroxidase C. Eur J Biochem 96:483–502