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Genetics of the peroxidase isoenzymes in *Petunia*

6. Differential temporal expression of *prxB* alleles

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Summary. In *Petunia* four alleles of the gene *prxB* could be identified by starch gel electrophoresis. Investigation of PRXb allozyme balance during development of tissue and plant of *prxB* heterozygotes showed differential temporal expression of the four mobility alleles. Analysis of developmental allozyme balance of *prxB2/B3* heterozygotes from F1 and F2 progenies indicated the presence of a trans-acting regulatory gene termed *Rp1*. Segregation for the gene Rp1 indicated monogenic inheritance. Independent segregation of the genes *Rp1* and *prxB* was found. When homozygous and recessive, the gene *Rp1* speeds up the start of expression in enzyme activity of the *prxB2* allele. The alleles *prxB1* and *prxB4* are expressed later in development than the alleles *prxB2* and *prxB3,* no matter the *Rp1* genotype. The final expression level of the *prxB* alleles is different for all four alleles, irrespective of the *Rp1* genotype.

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

Introduction

Mutants that show an aberrant temporal expression in enzyme activity can serve as material in studying regulation of gene expression. Such mutants can easily be scored by determining allozyme balances. Two of the conditions needed are the availability of a staining method to visualize enzyme activity on gels, and the availability of mobility alleles. The first reports using this approach were on the alcohol dehydrogenase system in maize by Schwartz (1966) and Schwartz and Endo (1966). Since then the number of papers dealing with allozyme balance in higher eukaryotes has increased steadily (Paigen 1979, 1980; Scandalios 1979).

As to allozyme balance studies in higher plants, much attention has been paid to the alcohol dehydrogenase system (Karoly et al. 1982) and the catalase system (Scandalios 1979; Sorenson 1982) in maize, and the peroxidase system in rice (Chu 1967; Shahi et al. 1969; Pai et al. 1973; Endo 1971, 1981) and petunia (van den Berg and Wijsman 1981, 1982a, b; van den Berg et al. 1982, 1983; Wijsman 1983).

A difference in activity of allozymes may be the result of a regulatory mutation. These mutations act in cis, and may be located in the regulatory part of the gene that codes for the allozymes. It may be that the same mutation of the structural part of the gene that causes a change in electrophoretic mobility of the allozyme is also responsible for a difference in activity of the allozymes. However, temporal programming mutations are more likely to be located in the regulatory part of the gene.

Analysis of plants from F2 progenies that are heterozygous for the mobility mutation can reveal, in addition to the cis-acting mutation, the presence of mutations that act in trans; but only when the mobility alleles differ in responsiveness to the signal from the trans-acting gene, caused by the cis-acting mutation.

Thus far, most papers dealing with allozyme balances report on cis-acting mutations (Paigen 1979, 1980). Trans-acting genes have been reported for the catalase system in maize (Scandalios et al. 1980) and the peroxidase system in rice (Endo 1981).

In a previous publication we reported on the developmental expression and location of the structural gene *prxB* (van den Berg and Wijsman 1982a). Here, we report on the presence of four internal site differences of the gene *prxB* in *Petunia.* Furthermore, crossing experiments indicated the presence of an external site

mutation that particularly affects expression of the allele *prxB2* as could be shown in *prxB2/B3* heterozygotes.

Materials and methods

Plant material

The following inbred lines were used: *P. axillaris* ssp. *axillaris*: S1; *P. integrifolia* ssp. *inflata:* S6 and S9; (the origin of lines S1, S6 and S9 has been given earlier, van den Berg and Wijsman 1982a); *P. hybrida:* R51, V23, M1, W4 and V42 (derived from the cultivars 'Royal Ruby', 'Blauzwerg', 'Rose of Heaven', 'Pendula Cyanea' and 'Deep Blue', respectively, as well as W22 (derived from the cultivars 'Brick-red' and White Cloud') and V55 (line of recombinant descent). Furthermore, the following plants were used: *P. hybrida:* E7721D1 (derived from the progeny of (trisomic III×V23)×S9); *P. integrifolia* ssp. *inflata:* D580-2, D582-1, D582-2, family E7273 (36 plants); *P. integrifolia* ssp. *integrifolia:* D579-1, D579-2, D659-3, D660-1, D660-2. The origin of the *P. integrifolia* sl. plants has been given by van den Berg and Wijsman (1982b) (Family E7273 has the same origin as D580-2).

Electrophoretic analysis of the peroxidase isoenzymes

Sample preparation, electrophoretic separation of the peroxidase isoenzymes using gel system I, staining for peroxidase activity, and densitometric scanning of zymograms were as described previously (van den Berg and Wijsman 1981, 1982a, b).

Nomenclature of the peroxidase genes and enzymes

Nomenclature of the peroxidase genes and enzymes, and the definition of internal and external site mutation as given previously is followed here (van den Berg and Wijsman 1981, 1982a, b). Trans-acting genes that regulate expression of peroxidase genes are given the symbol *Rp* (Regulatory gene for peroxidase) and are numbered.

Results

The prxB1, prxB3 and prxB4 internal site mutations

Among our inbred lines of *Petunia* the allele *prxB1* was found in line S1 (*P. axillaris* ssp. *axillaris*). Analysis of the PRXb developmental allozyme balance of the F1 (S1×R51) (genotype *prxB1/B2*) showed that the *prxB1* temporal programme differs from that of the standard allele *prxB2* from line R51 (van den Berg and Wijsman 1982a).

The progenies of the crosses F2 (S1×R51) and (trisomic I×S1)×R51 (genotype *prxB1/B2/B3*×*prxB2/B2*) were investigated to see whether there are external site differences between the lines S1 and R51. All *prxB1/B2* heterozygotes of both progenies showed a lower activity of the PRXb1 enzyme in leaf tissue of young plants and a higher activity in old flowering plants. In young leaves of young plants expression of the *prxB2* allele starts earlier. We conclude that the difference in the temporal expression of the alleles is caused by an internal site mutation and that there are no detectable external site differences between lines S1 and R51 (Fig. 1).

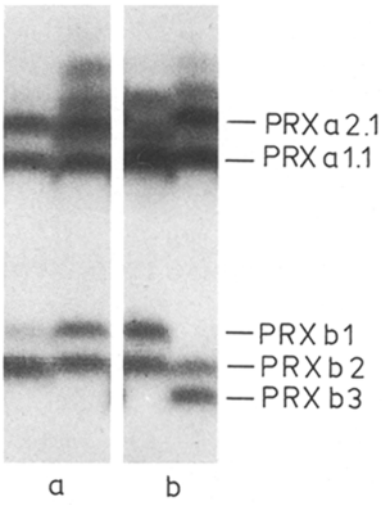

Fig. 1a, b. Starch gels showing differential expression of *prxB* alleles in the progeny of trisomic I×R51 (genotype: *prxB1/B2/B3*×*prxB2/B2*). **a** Analysis of young (*left*) and mature (*right*) leaf from a young plant with genotype *prxB1/B2* at the onset of flowering. Note a lower activity of *PRXb1* in young tissue and a higher activity in older tissue, compared to *PRXb2*. **b** Analysis of mature leaves from flowering plants with genotype *prxB1/B2* (*left*) and *prxB2/B3* (*right*)

The allele *prxB3* was found in lines S6 and S9 (*P. integrifolia* ssp. *inflata*). Analysis of young leaves of

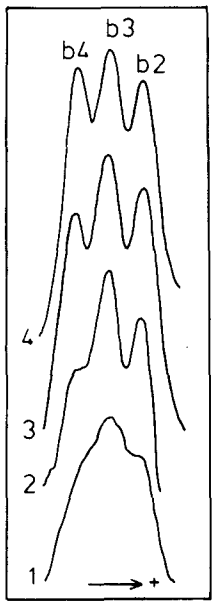

Fig. 2. Densitometric scannings of a starch gel showing differential expression of the alleles *prxB2, prxB3,* and *prxB4* in a triply heterozygous trisomic I. Young to old leaves (*1* to *4*) were taken from a young flowering trisomic

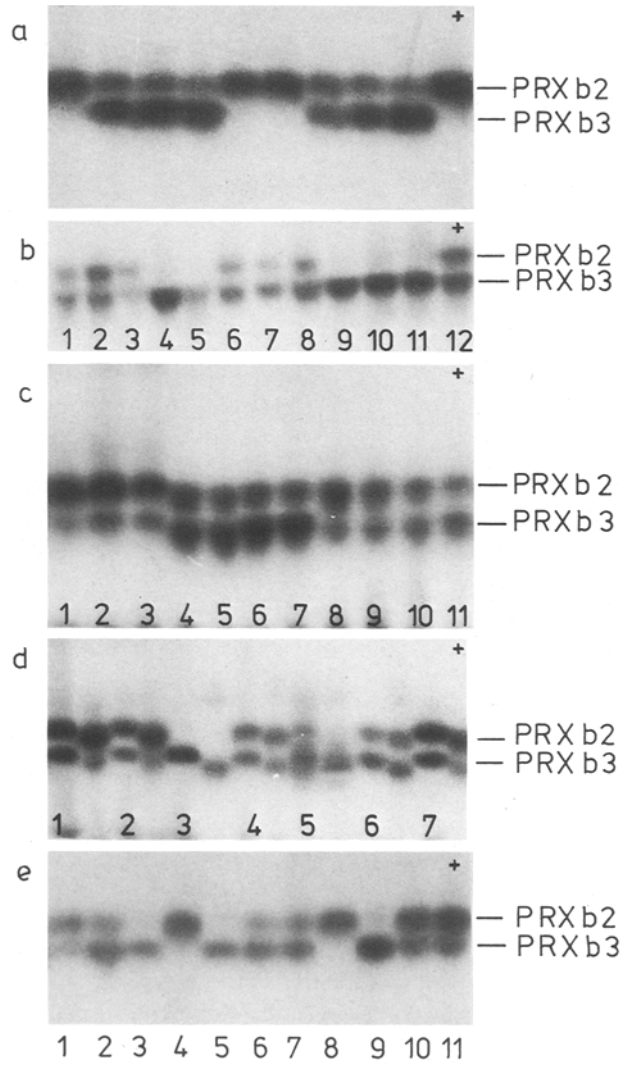

Fig. 3a–e. Starch gels showing differential temporal expression of the alleles *prxB2* and *prxB3*, and segregations for the trans-acting regulatory gene *Rp1*. **a** Analysis of mature leaves from flowering plants of the progeny of (W22×V42)×M1 (genotype *prxB2/B3*×*prxB2/B2*). All *prxB2/B3* heterozygotes have *PRXb2* about half as active as *PRXb3*. **b** Analysis of young leaves from young plants of the progeny of B1 (W4×S6)×S6 (genotype *prxB2/B3,Rp1rp1*×*prxB3/B3, rp1rp1*). Nos. *1, 6, 8:* genotype *prxB2/B3,Rp1rp1*; nos. *2, 3, 12: prxB2/B3,rp1rp1*; nos. *4, 5, 7, 9–11: prxB2/B2.* **c** Analysis of leaves of different age (*left* to *right:* young to old) from three flowering plants of the progeny of B1(W4×S6)×S6. Plant 1 (nos. *1–3*): *prxB2/B3,rp1rp1;* plant 2 (nos. *4–7*): *prxB2/B3,Rp1rp1;* plant 3 (nos. *8–11*): *prxB2/B3,rp1rp1*. Note the difference between plant 1 and plant 3. Though they are both *rp1rp1,* plant 1 has a higher PRXb2, whereas plant 3 has a lower PRXb2 activity in older leaves of the flowering plant. **d** Analysis of young and mature leaves from young plants of the progeny (E7721D-1×D660-1)×(D582-1×D579-1) (genotype *prxB2/B3,Rp1rp1*×*prxB2/B3,Rp1rp1*). The numbers refer to two lanes of which the *left* lane represents analysis of young leaf and *right* lane mature leaf of the plant. The *prxB3* and *rp1* allele of the parent derived from the cross E7721D-1× D660-1 originates from line S9. Nos. *1, 2, 4, 6, 7* have genotype *prxB2/B3,rp1rp1;* no. *3* has genotype *prxB3/B3;* no. *5* has genotype *prxB2/B3,Rp1rp1* or *Rp1Rp1*. **e** Analysis of young leaves of young plants of the progeny E7721D-1×E7711C-69 (genotype *prxB2/B2,Rp1rp1*×*prxB2/B3, Rp1rp1*). Nos. *1, 10, 11* have genotype *prxB2/B3,rp1rp1;* nos. *3, 4, 5, 8, 9* are homozygous for *prxB;* nos. *2, 6, 7* have genotype *prxB2/B3, Rp1Rp1* or *Rp1rp1*

young flowering plants of F1 (S6×R51) (genotype *prxB2/B3*) showed that the allele *prxB3* was expressed first. During further development, the initiation of expression of *prxB2* could be detected, and the difference in allozyme activity gradually became smaller.

When PRXb2 has reached an activity about half that of PRXb3, this difference persisted during further development. We conclude that line S6 contains an internal site mutation that causes the different temporal expression with regard to the standard allele *prxB2* of line R51. (See below for external site difference.) The *prxB3* allele was also found in lines S9 and S10 (*P. integrifolia* ssp. *inflata*) and line W22 (*P. hybrida,* derived from the cultivars 'Brick-Red' and 'White Cloud'). Crossing experiments showed that these lines contain the same allele as line S6 (Fig. 3, Tables 1, 2, and unpublished results).

In plants of *P. integrifolia* ssp. *integrifolia* obtained from seeds collected in Brazil (E7273), the enzymes PRXb2, PRXb3 and PRXb4 were found. The PRXb4 enzyme has the lowest electrophoretic mobility of the three. A plant from family E7273 with genotype *prxB2/B4* was crossed to a trisomic I with genotype *prxB2/B2/B3.* Among the progeny one trisomic I with genotype *prxB2/B3/B4* was found. The *prxB4* allele appeared to have another temporal programme as the alleles *prxB2* and *prxB3* (Fig. 2). The *prxB4* allele resembles the *prxB1* allele in that its expression starts later during development of the plant than the expression of the alleles *prxB2* and *prxB3*. But during further development the expression of the *prxB4* allele increases relatively. In mature leaves of the flowering trisomic the PRXb4 enzymes are more active than the PRXb2 enzymes. In contrast to the *prxB1* allele, the allele *prxB4* has in mature leaves of the flowering trisomic a lower expression level than the *prxB3* allele. We conclude that different temporal expression of the *prxB4* allele is caused by an internal site mutation.

Table 1. Segregation for the gene *Rp1* among *prxB2/B3* descendants involving *P. hybrida, P. integrifolia* ssp. *integrifolia* and ssp. *inflata,* and *P. axillaris* ssp. *axillaris plants*

Parents and their genotypes[a]		*prxB2/B3* descendants		Chi square tests for *Rp1* segregation	
		Rp1Rp1 *Rp1rp1*	*rp1rp1*	$X^2_{3:1}$	*P*
1.	(E7721D-1 × D660-1) selfing *prxB2/B3* × *prxB2/B3*	34	11	0.0074	0.93
2.	E7721D-1 × (E7721D-1 × D660-1) *prxB2/B2* × *prxB2/B3*	50	5	7.42	0.01
3.	E7721D-1 × E7711C-69 *prxB2/B2* × *prxB2/B3*	40	3	7.45	0.01
4.	E7721D-1 × (D579-1 × D582-2) *prxB2/B2* × *prxB2/B3*	34	6	2.13	0.13
5.	E7721D-1 × (D582-1 × D579-1) *prxB2/B2* × *prxB2/B3*	19	4	0.71	0.40
6.	(E7721D-1 × D660-1) × (D582-1 × D579-1) *prxB2/B3* × *prxB2/B3*	24	8	0	1.0
7.	E7721D-1 × (D579-2 × D579-2) *prxB2/B2* × *prxB2/B3*	25	5	1.11	0.29
8.	(D579-2 × S2) × (D579-2 × S2) *prxB2/B3* × *prxB2/B3*	19	4	0.71	0.40
9.	E7721D-1 × (S2 × D580-2) *prxB2/B2* × *prxB2/B3*	23	7	0.044	0.83

[a] All parents have genotype *Rp1rp1*

An external site mutation regulating prxB

Analysis of the PRXb2b3 allozyme balance during development of the F1 (W4 × S6) shows a difference in the temporal expression of the alleles *prxB2* and *prxB3* similar to that of the F1 (R51 × S6). Thus, we assume that lines W4 and R51 share the same *prxB2* allele. The *prxB2/B3* heterozygotes of the backcross (W4 × S6) × S6 progeny showed an aberrant type in addition to the parental developmental allozyme balance type (Fig. 3). In the parental type the *prxB2* allele was expressed latest, whereas in the aberrant type the *prxB2* allele was expressed earliest in development. In mature leaves of flowering plants of the aberrant type the difference in allozyme activity ranged from an activity of the PRXb2 enzymes of about half as active to about thrice as active as the PRXb3 enzymes (Fig. 4, "Discussion").

Further crossing experiments showed that an external site mutation is responsible for this aberrant developmental allozyme balance type. We assume that line S6 is recessive for the trans-acting gene, termed *Rp1,* and that line W4 is homozygous dominant for the gene. All *prxB2/B3* heterozygotes (24 plants) of the B1 (W4 × S6) × W4 showed differences in allozyme balance changes similar to those of the F1 parent. Absence of segregation for *Rp1* is in agreement with the postulated dominant *Rp1* genotype of line W4.

The gene *Rp1,* when present in the dominant form, delays the start of expression of the *prxB2* allele. In very young leaves of young not yet flowering plants with genotype *Rp1rp1* or *Rp1Rp1,* the PRXb2 enzyme can barely be detected. However, if the genotype is *rp1rp1,* the PRXb2 enzyme can easily be detected in young tissue. Such a strong influence of *Rp1* on the

Table 2. Genotype of *P. hybrida, P. axillaris* ssp. *axillaris, P. integrifolia* ssp. *integrifolia* and ssp. *inflata* plants with respect to the genes *prxB* and *Rp1*

P. axillaris ssp. *axillaris*	S2	*Rp1Rp1*	*prxB2/B2*
P. integrifolia ssp. *inflata*	S6	*rp1rp1*	*prxB2/B2*
	S9	*rp1rp1*	*prxB3/B3*
	D580-2	*rp1rp1*	*prxB3/B3*
	D582-1	*rp1rp1*	*prxB2/B3*
	D582-2	*rp1rp1*	*prxB2/B3*
P. integrifolia ssp. integrifolia	D659-3	*Rp1Rp1*	*prxB2/B3*
	D660-1	*Rp1Rp1*	*prxB2/B3*
	D660-2	*Rp1Rp1*	*prxB3/B3*
	D579-1	*Rp1Rp1*	*prxB3/B3*
	D579-2	*Rp1rp1*	*prxB3/B3*
P. hybrida	R51	*Rp1Rp1*	*prxB2/B2*
	W4	*Rp1Rp1*	*prxB2/B2*
	V23	*Rp1Rp1*	*prxB2/B2*
	V55	*Rp1Rp1*	*prxB2/B2*

timing of expression of the *prxB3* allele was not apparent. The presence of a dominant *Rp1* allele in *prxB2/B3* plants can easily be determined by the PRXb2b3 allozyme balance of young leaves from young plants.

The genes prxB and Rp1 in P. integrifolia ssp. integrifolia and ssp. inflata

Genotypes of *P. integrifolia* s.l. inbred lines, and plants obtained from Argentina and Brazil with respect to the genes *prxB* and *Rp1* are summarized in Table 2. Relevant crosses are given in Table 1.

Of line S9 (*P. integrifolia* ssp. *inflata*), 6 old plants were available with genotype *prxB2/B3*. They all showed a higher activity of the PRXb2 enzyme in mature leaves. Therefore, we assume that line S9 has genotype *rp1rp1*. This assumption was confirmed by the observation of segregation for *Rp1* in the progeny obtained from a cross of a plant from D7656P-28 × S9 (genotype *prxB2/B2, Rp1Rp1* × *prxB3/B3, rp1rp1*) to a plant from B1 (W4 × S6) × S6 (Table 1). The latter plant had genotype *Rp1rp1*, and the former plant had the same genotype, the recessive *Rp1* allele originating from line S9.

Plants D582-1 and D582-2 (*P. integrifolia* ssp. *inflata*) appeared to have genotype *prxB2/B3, rp1rp1*, as judged by the PRXb phenotype. Crossing of both plants to a plant with one or two dominant *Rp1* alleles showed that they also contain the *prxB3* internal site mutation. We assume that the *prxB3* allele involved is identical to the allele from line S6. Also, plant D580-2 (*prxB3/B3, P. integrifolia* ssp. *inflata*) appeared to have the same *prxB3* alleles as line S6, and the genotype for the gene *Rp1* was *rp1rp1*.

Plants D659-3 and D660-1 (*P. integrifolia* ssp. *integrifolia*) had genotype *prxB2/B3*. They showed developmental allozyme balance changes similar to the F1 (R51 × S6). Plants D660-2, D579-1 and D579-2 (*P. integrifolia* ssp. *integrifolia*) had genotype *prxB3/B3*. Crossing experiments showed that all *prxB3* alleles involved can be assumed to be identical to the *prxB3* alleles from lines S6 and S9, and that all plants are homozygous dominant for the gene *Rp1*.

Discussion

In the present paper differential temporal expression of four alleles of the gene *prxB* is described. Differential expression was not only apparent by differences in increase of PRXb activity during development of tissue, but also by differences in start of expression.

Except for mutation of the gene *Rp1* no external site differences that clearly influence temporal expression of the gene *prxB* were found between the plants used. As argued in a former paper on differential temporal expression of *prxA* alleles (van den Berg et al. 1983), other external site differences may have escaped our attention, because allozyme balance studies do not reveal the presence of external site mutations that influence expression of both alleles of a heterozygote in the same way.

The genotype for the gene *Rp1* could be recognized by the allozyme balance of *prxB2/B3* plants in young leaves. In plants with genotype *rp1rp1* the *prxB2* allele is expressed first. In old and mature leaves of flowering plants the PRXb2 activity ranges from about half as active to about thrice as active as that of PRXb3 (Fig. 4). This range in allozyme balance may indicate the presence of other genetic factors influencing expression of the gene *prxB*.

Segregation for the gene *Rp1* was found among several progenies. The results presented (Table 1) indicate that most of the segregations found do not deviate significantly from a 3 : 1 segregation (though there is a slight surplus of dominant alleles among most progenies). This indicates complete dominane of the *Rp1* allele. We may conclude that there is no reason to assume linkage between the genes *Rp1* and *prxB*.

The observed segregations for the gene *prxB* among most of the progenies analysed deviated significantly from the expected backcross or F2 segregation (data not shown). In addition to results of other crossing

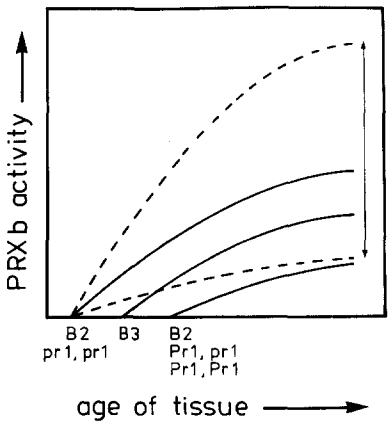

Fig. 4. Diagrammatic presentation of differential temporal expression of the alleles *prxB2* and *prxB3* in plants (homozygous) recessive or dominant for the trans-acting regulatory gene *Rp1*. Genotypes for *prxB* and *Rp1* are indicated on the diagram. The diagram is not based on measurements of enzyme activity, but is only based on allozyme balance studies. It serves to illustrate the difference in start of expression of the *prxB2* and *prxB3* alleles, and differences in increase of PRXb activity during aging of tissue. For the *B2,rp1rp1* curve the range of activities is indicated with two dashed curves and arrows. The *B3* curve is apparently (see text) the same for plants homozygous dominant or recessive for the gene *Rp1*

experiments involving *P. hybrida* and its progenitor species (van den Berg and Wijsman 1982a), these results may be explained by the presence of deleterious mutations that influence transmission to the progeny of *prxB* alleles linked to those mutations. Among the progeny of (V42×W22)×M1 (genotype *prxB2/B3, hf1Hf1×prxB2/B2, hf1hf1;* 126 plants) no crossing-over was found between *Hf1* and *prxB*, whereas among the progeny of F2 (S6×R51) 16% crossing-over was found (van den Berg and Wijsman 1982a). Thus, we must be very cautious with conclusions as to genetic linkage among progenies that have been obtained by crossings of *P. hybrida* to its putative progenitor species.

It may be that two internal site mutations are involved in differential expression of the alleles *prxB2* and *prxB3*. One that causes differential expression in the absence of the trans-acting signal form *Rp1*, and the other that causes a difference in responsiveness to the trans-acting signal.

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