Sn, a Light-Dependent and Tissue-Specific Gene of Maize: the Genetic Basis of Its Instability

Giuseppe Gavazzi, Mariangela Mereghetti, Gabriella Consonni and Chiara Tonelli

Dipartimento di Genetica e di Biologia dei Microrganismi, Università Degli Studi di Milano, via Celoria 26, 20133 Milano, Italy

Manuscript received October 31, 1988
Accepted for publication January 15, 1990

ABSTRACT

The genetic system under investigation is defined by three major components: a gene, *Sn*, conferring tissue specific anthocyanin accumulation in different plant regions, light, required for color development in competent tissues, and another gene, *Pl*, substituting for light in its capacity to elicit pigment production. Attention is given in this paper to an *Sn* allele, symbolized *Sn:bol3*, capable of some constitutive pigmentation in seedlings and seed integuments. *Sn:bol3* confers a higher pigment potential than the other alleles and is unstable. Its instability relates to its frequent changes from an original condition, indicated as *Sn-s*, to *Sn-w*, where *-s* and *-w* stand for strong and weak and refer to the two levels of seedling pigmentation. Weak derivatives arise spontaneously at a high frequency in homo- and heterozygous *Sn:bol3* genotypes. In the latter, weak derivatives are also recovered on the chromosome originally devoid of *Sn* as if the heterozygous association had promoted "contamination" of one chromosome (recipient) with *Sn* coming from the other (donor). If the two chromosomes in the heterozygote are marked with contrasting alleles of *R*, a gene lying about two crossover units proximal to *Sn*, it appears that the *R* constitution of the recipient chromosome affects their constitution. Presence of *R-r* in fact leads to changes of both chromosomes in terms of *Sn* constitution, resulting in a majority of nonparental chromosomes, *R-r Sn* and *r Sn-w* or *r sn*, while replacement of *R-r* with *R-g*, a mutant derivative of *R-r*, leads to a drastic reduction in the yield of nonparental chromosomes. This unexpected result could suggest a transactive effect of *R-r* upon the rate of passage of *Sn* from one chromosome to the other or, alternatively, it could be explained by assuming that in heterozygous *Sn/sn* plants *Sn* activates a cryptic *Sn* residing on the other homologue and that the presence of *R-r* is instrumental in inciting this activation process.

FLAVONOID biosynthesis presents unique features that make it an appealing model system for the analysis of gene regulation as well as for studies in developmental biology. This process in fact requires many genes whose gene action has been identified (COE and NEUFFER 1977) and is modulated by exogenous factors such as light and hormones (NAGY, KAY and CHUA 1988). In addition, besides the genes directly involved in the pathway, other known genes play a regulatory role by controlling the amount of pigment and its distribution.

An example of these is *Sn*, a gene accounting for tissue specific accumulation of anthocyanin in maize (GAVAZZI *et al.* 1986).

Several *Sn* sources have been identified. They all map on the long arm of chromosome *10*, about two map units distal to *R* (*R* being one of several genes required for pigment accumulation in different plant tissues) and appear distinguishable by differences in the rate of accumulation and in the level of pigmentation. *Sn* is light dependent in its expression. Tissues devoid of pigment, but developing color in the presence of *Sn*, are the scutellar node, mesocotyl, leaf base and midrib and ovary integuments (glumes and pericarp). The latter tissues, however, show constitutive pigment production if *Pl*, an unlinked factor lying on chromosome *6*, is present in their genome.

Previous studies on the activity of different enzymes of flavonoid biosynthesis in young seedlings differing in their *Sn* constitution and grown either in darkness or in continuous light, have shown that *Sn* increases the level of these enzymes in a coordinate, light dependent manner suggesting a transactive regulatory role of this gene on the activity of the structural genes involved in flavonoid biosynthesis (GAVAZZI *et al.* 1985b). In addition, irradiation with light of different qualities of tissues (pericarp or mesocotyl) of *Sn* lines where the gene is expressed has indicated that blue light elicits the strongest effect in terms of pigment quantity, thus suggesting a link between *Sn* and the presence of a photoreceptor activated by blue light (GAVAZZI *et al.* 1985a).

In this paper we describe the genetic instability of an *Sn* allele symbolized *Sn:bol3*. Data will be presented disclosing a functional interdependence between *Sn* and *R*. The instability under investigation, an intrinsic property of *Sn:bol3*, is influenced by the *R* constitution of the chromosome devoid of *Sn*, as revealed by analysis of appropriate heterozygous combinations.

Among the *R sn/r Sn:bol3* heterozygotes differing in their *R* constitution, those carrying *R-r* (a compound allele consisting of two adjacent genes, symbolized *P* and *S*, that control color distribution in plant and seed respectively) yield, on testcrossing, a large majority of nonparental progeny seedlings showing loss of *Sn* from the parental *r Sn* chromosome and gain of *Sn* in the *R* marked chromosome. These results are not observed in heterozygotes carrying *R-g* or *R-st*. Thus *R-r* specifically confers *Sn* instability presumably through a trans-active signal leading to passage of *Sn* from one homolog to the other or alternatively through activation of a silent *Sn* gene residing on the *R-r* marked chromosome.

MATERIALS AND METHODS

Description of chromosome *10* markers: *The R locus*: detailed description of the origin, phenotype and structural characteristics of the *R* alleles used in this study can be found in DOONER and KERMICLE (1974, 1976). Properties of the *R* alleles that are pertinent to this presentation are outlined below.

R-r:standard: purple aleurone, red plant parts. A gene complex, consisting of two gene components, symbolized (*P*) and (*S*) controlling plant and seed color, respectively.

R-g: purple aleurone, green plant. An (*S*) derivative of *R-r* that molecularly appears devoid of the (*P*) component (C. TONELLI, S. CONSONNI, S. DELLA PORTA and G. GAVAZZI, unpublished data).

R-st: stippled aleurone, green plant. A pattern allele of *R* consisting of dark spots in the aleurone-Paramutagenic. Component constitution is consistent with the model (*Sc*) (*I-R*) (*Nc*) (ASHMAN 1970, KERMICLE 1984).

R-sc: self colored aleurone, green plant (KERMICLE 1970). A germinal derivative of stippled showing the same pattern of *R-st* at the molecular level (C. TONELLI, S. CONSONNI, S. DELLA PORTA and G. GAVAZZI, unpublished data).

r-g: colorless aleurone, green plant. The null allele.

The Sn locus: a factor lying two map units distal to *R*, conferring specific tissue pigmentation to the scutellar node, mesocotyl, leaf basis and midrib and to the seed integuments (glumes and pericarps) following light exposure. Three independent *Sn* accessions (*bol1, bol2, bol3*) have been identified in separate Bolivian populations collected by G. AVILA (Centro Fitotecnico de Pairumani, Cochabamba) and a fourth one (*Sn:co-op*) in a stock kindly provided by the Co-op center. They were transferred to a common genetic background by repeated backcrosses into a colored version of the W22 inbred line. All four accessions are borne on a *r* marked chromosome and confer weak red pigmentation to the anthers. To establish whether this character is *r* or *Sn* dependent, progeny of *R-sc Sn/r-g sn* parents were scored for anther pigmentation. 71 out of 73 such derivatives lost their pigmenting capacity in these tissues, a result consistent with the hypothesis that the resident *R* allele of the *r Sn* accessions is of the *r-r* (*P s*) type. A more direct proof of its constitution based on the lack of anther pigmentation in the presence of the reciprocal class of crossover derivatives (*r sn*) has not been obtained because this class of recombinants has so far not been recovered (see RESULTS).

Lc is another factor that overlaps in its tissue specific expression with *Sn* but differs in its characteristic leaf pigmentation (DOONER and KERMICLE 1976). This factor was extracted from an Ecuadorian strain and maps 1.5 units distal to R. It might well be that *Sn* and this factor represent different geographic isolates of the same gene but no genetic evidence of their allelism has so far been obtained due to the poor expression of *Lc* in Northern Italy climate conditions. In this study two *Sn* accessions have been considered. one, *Sn:bol2*, used as a control, confers a uniform mesocotyl pigmentation and is stable in its expression. The other, *Sn:bol3*, confers a deep red mesocotyl pigmentation, about five times stronger than that of *bol:2*, and differs from *Sn:bol2* and the other accessions in its instability.

Progeny of homozygous *r Sn:bol3* parents selected at the seedling stage for presence of a strong red mesocotyl pigmentation consist of seedlings with the parental phenotype, referred to as *Sn-s*, as well as of seedlings with a significantly lower pigment level ranging from almost colorless to fully colored, and collectively referred as *Sn-w* derivatives. Variants selected as *Sn-s* at the seedling stage show light independent seed integument pigmentation, while *Sn-w* variants are devoid of pigment in these tissues. The varying degrees of pigmentation of the weak derivatives can be selected for suggesting a genetic basis for the observed differences (GAVAZZI *et al.* 1985). Their rate of occurrence is variable, generally accounting for 15–30% of the progeny obtained by selfing homozygous *r Sn-s* plants. *Sn-s* selections continue to reveal instability in succeeding generations while *Sn-w* selections never revert to the *Sn-s* phenotype. No instability of the kind here described has been observed with *Sn:bol2*, *Sn:bol1* and *Sn-Co:op*.

R-st Sn:bol2 derivatives are recovered in the progeny of *R-st sn/r Sn:bol2* parents test crossed with *r-g sn* lines. If the heterozygous parents carry appropriate flanking markers, the *R Sn* derivatives recovered in the progeny are associated with outside marker recombination.

R-sc Sn:bol3 derivatives (*Sn-s* selections): these also arise through recombination, like the *R-st Sn:bol2* derivatives. They differ from the latter in their instability, giving a variable yield of *-s* and *-w* variants in their progeny.

M-st: a dominant intensifier of the spotting pattern of *R-st*, mapping six units distal to *R*. Its effect increases with dosage and is transactive.

O7: opaque endosperm, 26 map units distal to *R*.

Germination: Seeds were surface-sterilized with 5% Orthocyde for 24 hr on a rotary shaker, rinsed with sterile distilled water and transferred to plastic boxes with wet filter paper (Whatman No. 3) below and above the seeds. They were then allowed to germinate in darkness for 5 days at 25 ± 2° until a mesocotyl about 8 cm long developed. Seedlings were then exposed to continuous white light for 70 hr at 21°. As a light source Power Stars-HQI-T400 W/DV OSRAM lamps were used with fluence rate of 68 $W \cdot m^{-2}$. At the end of the irradiation time seedlings were scored for their mesocotyl pigmentation level on a visual basis and classified as strong, weak, and nonred. In some experiments pigmentation level was determined by anthocyanin extraction from individual excised mesocotyls.

Light treatments on immature seeds: Immature ears at different days after pollination (DAP) were surface-sterilized with 7% chlorox for 20 min, rinsed with sterile distilled water, cut longitudinally into two halves, and placed in plastic boxes layered with 0.9% agar.

They were then irradiated with continuous white light for 96 hr at 21°. Controls were kept in darkness in the same conditions. At the end of the irradiation seeds were excised, their pericarps removed, and anthocyanins individually extracted from seeds and their corresponding pericarps.

Anthocyanin extraction: Anthocyanins were extracted with a fixed volume of 1% HCl in ethanol, the extracts

TABLE 1

Number and presumed constitution of chromosomes recovered in the progeny of *R-st sn/r Sn:bol2* and *R-st Sn:bol2/r sn* females pollinated by *r-g sn* males

Genotype	Population	Chromosome constitution				*P*(%)
		R-st sn	*r Sn*	*R-st Sn*	*r sn*	
R-st sn / *r Sn*	5772	2901	2801	69	0	2.3[a]
		R-st Sn	*r sn*	*R-st sn*	*r Sn*	
R-st Sn / *r sn*	5969	2876	2862	107	124	3.9

R-st Sn:bol2 is a recombinant derivative isolated in the progeny of plants with the first genotype.

[a] Percent of *R-st Sn* nonparentals over total number of *R-st* marked chromosomes.

centrifuged twice and absorption determined spectrophotometrically at 530 nm.

Anthocyanin concentration is expressed as Absorbance value at 530 nm per mg of fresh weight or per organ (pericarp or seed without pericarp).

RESULTS

Nonreciprocal recombination: Each of the *Sn* alleles so far tested was borne on an *r* marked chromosome. If heterozygotes carrying *r Sn* on one homolog and a contrasting *R* allele on the other are reciprocally crossed to an *r-g sn* stock only one of the two complementary classes of recombinants, *i.e.*, *R Sn*, is recovered. However, if heterozygotes carrying the recombinant chromosome, *R Sn/r-g sn*, are pollinated by *r-g sn* males the two reciprocal classes of recombinants are observed with approximately equal frequency in their progeny. This unexpected pattern of recombination, exemplified in Table 1 with the *Sn:bol2* source, is common to all *Sn* alleles so far tested and could be explained on the basis of different hypotheses as illustrated in Figure 1 (see DISCUSSION).

Instability of *Sn:bol3*: The *Sn:bol3* accession differs from the others in that it confers a higher pigmentation level in the mesocotyl and seed integuments, even in the absence of light, and in its instability. Two independent observations describe the phenomenon of instability. On the one hand the term refers to the frequent yield of derivatives in the progeny of homozygous *Sn:bol3* stocks that differ from the parental variant by a significant reduction in their mesocotyl pigmentation level. They are collectively referred to as weak derivatives (*Sn-w*) while the parental form is indicated as *Sn-s* (*-s* and *-w* standing for strong and weak pigmentation respectively). The change of *Sn-s* to *Sn-w* is generally unidirectional. Reversion can, however, be induced by treatment of *Sn-w* seeds with the deoxycytidine analog 5-aza-2′-deoxycytidine, thus suggesting an involvement of DNA modification in *Sn* expression (TONELLI *et al.* 1988). Continuous selfing of homozygous *r Sn-s* selections shows a constant

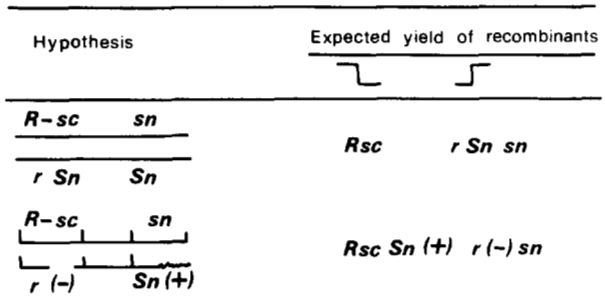

FIGURE 1.—Two alternative hypotheses to explain nonreciprocal recombination. The first assumes that the *r Sn* chromosome carries two separate *Sn* genes, one closely linked to *R* and the other about two map units distal to *R*. The second hypothesis assumes that *r* is missing a function (−) essential for its gametic transmission and complemented by *Sn*(+).

TABLE 2

Yield (%) of *R Sn* nonparental chromosomes recovered in the progeny of *r Sn:bol3/R sn* females pollinated by *r-g sn* males

Parental genotype	Population	*R Sn* nonparentals	
		S	*W*
r Sn:bol3 / *R-sc sn*	3175	1.32	23.09
r Sn:bol3 / *R-st sn*	4721	1.25	15.97

S and *W* stand for strong and weak mesocotyl pigmentation.

pattern of instability, with a large majority of progeny plants maintaining the parental phenotype (*i.e.*, strong red mesocotyl and light-independent seed glume pigmentation) and a minority of derivatives, generally about 25%, with weakly colored mesocotyl and light-dependent seed glume pigmentation. We refer to this pattern of events as replicating instability.

On the other hand, the term instability is also used to describe the frequent appearance in the progeny of *Sn-s/sn* individuals of weak derivatives bearing *Sn-w* on the chromosome originally devoid of *Sn*, as if association of the two in the heterozygous parent resulted in frequent passages of *Sn-w* from one chromosome to the other. This event is illustrated in the data of Table 2 where the progeny of *r Sn-s/R sn* heterozygotes receiving a nonparental *R Sn-s* chromosome are found at a rate expected from recombination (1–2%), while the *R Sn-w* progeny far exceeds that value. The former are in fact the result of crossing over in the *R-Sn* segment while the latter represent a different event since they are not associated with outside marker recombination.

This point is illustrated in the results of three point testcrosses (Table 3) using *O7* as outside marker (*O7* lying 26 cM distal to *R*) where the progeny of *R-st Sn/r sn* females show that the *r Sn* strands carrying the parental outside marker for *r* (*r Sn o7*), inferred to be double crossovers, are recovered with even greater frequency than the single recombinants (*r Sn o7*). In

addition, the segregation ratios for *R* and *Sn*, as observed in testcrossed progenies of such heterozygotes, are different. *R-r* in fact segregates as expected (1:1), while the *Sn:sn* segregation values exhibit an excess of *Sn* over *sn* (Table 4).

These unexpected features, *i.e.*, passage of *Sn* from one chromosome to its homolog and a distorted segregation ratio for *Sn*, are not observed if the heterozygous genotypes carry other *Sn* sources on the donor chromosome.

These data taken together seem to indicate an overreplication of *Sn* and its frequent passage from one homolog to the other. These events are referred to as transactive instability to distinguish it from replicating instability. Both kinds of instability are observed only with the *Sn:bol3* accession.

Effect of the *R* constitution on *Sn* instability: An intriguing and unexpected result is the effect of the *R* constitution of a normal chromosome 10 on the *Sn* instability of its homolog. If pollen of an *r Sn-s* line is used to pollinate *R-r sn* and *R-g sn* stocks to develop two genotypes sharing the *r Sn-s* chromosome but differing in the constitution of the other homolog, the outcome in the progeny of the two heterozygotes is strikingly different. *R-g sn/r Sn-s* females pollinated by *r-g sn* males yield, as expected, two preponderant progeny classes (seedlings receiving either one of the two parental chromosomes) and only one nonparental class consisting of *R-g Sn-s* or *R-g Sn-w* seedlings. The results observed with *R-r sn/r Sn-s* females, on the other hand, differ greatly. Instead of the parental *R-r sn* and *r Sn-s* chromosomes, the progeny obtained by testcrossing these heterozygotes consists of nonparental chromosomes only: *R Sn-s* or *R Sn-w*, on the one hand, and *r sn* or *r Sn-w*, on the other. All four classes appear at about the same frequency. The data pertaining to this point are given in Table 5 together with the results obtained in the progeny of *R-st sn/r Sn-s* heterozygotes. The latter genotype is included in the Table to emphasize the fact that the recovery of a majority of nonparental chromosomes in the progeny of heterozygous plants is observed only when *R-r* is employed as contrasting allele.

"Transfer" of *Sn* from one chromosome to its homolog: To establish whether the *Sn* "transferred" from one homolog to another resides at the same location in the homolog receiving it, two independent cases of "transfer" originating from *R-sc sn/r Sn:bol3* and *R-r sn/r Sn:bol3* respectively were studied. Heterozygous *R Sn +/r-g sn M-st* females (where *R* stands for *R-sc* or *R-r* and the symbol + stands for absence of *M-st*) were mated to *R-st sn* + males and recombinants in the *R-M-st* interval detectable as light stippled *vs.* parental stippled kernels, were isolated. They represent only one of the two reciprocal classes of recombinants, *r-g+*, expected from *R +/r-g M-st* heterozygotes, since the second class, *R M-st*, is not detectable in this cross.

TABLE 3

Effect of the *Sn* source (*bol2 vs. bol3*) on the results of three point testcrosses of *R-st Sn O7/r sn o7* females with *r sn o7* males

Progeny classes			Inferred strand constitution			Frequency ($\times 10^{-2}$)	
						Sn:bol2	*Sn:bol3*
R	*Sn*	*O7*	*R*	*Sn*	*O7*	40.7	29.0
r	*sn*	*o7*	*r*	*sn*	*o7*	38.9	27.6
R	*sn*	*o7*	*R*	*sn*	*o7*	1.3	1.1
r	*Sn*	*O7*	*r*	*Sn*	*O7*	1.8	6.1
R	*Sn*	*o7*	*R*	*Sn*	*o7*	7.5	9.1
r	*sn*	*O7*	*r*	*sn*	*O7*	9.1	11.9
R	*sn*	*O7*	*R*	*sn*	*O7*	0.4	2.3
r	*Sn*	*o7*	*r*	*Sn*	*o7*	0.2	12.8
No. seedlings						5969	3889

Sn-s and *Sn-w* derivatives observed when *Sn:bol3* is analyzed are pooled together to make *Sn:bol3* vs. *Sn:bol2* results comparable.

A sample of the light stippled kernels was progeny tested to verify its *Sn* constitution as well as to ascertain the position of the transferred *Sn* in the *R-M-st* chromosome interval. Since the results obtained with the two *R Sn* derivatives are not significantly different they are considered together. Out of 38 verified light stippled recombinants 11 gained *Sn* and 27 were *sn* in phenotype. Since the observed frequency of light stippled recombinants is 5.07% (125/2465), the position of the transferred *Sn* can be inferred to be 1.47 cM distal to *R*, a value similar to the map position of *Sn*. These results could indicate that the "transferred" *Sn* from one homolog to another resides at the same location in the homolog receiving it.

Functional relatedness of *R* and *Sn*: Even though *R* and *Sn* are separate chromosomal components there is evidence suggesting their functional interdependence.

The evidence comes from the following observations: (1) A comparison of the kinetics of pigment accumulation in the aleurone (a tissue of *R-sc* competence) of homozygous *R-sc Sn-s* and *R-sc sn* stocks discloses a significant increase of pigment level in the former, particularly evident at 28 DAP, provided the immature seeds are kept in darkness (Figure 2). In the pericarp, a tissue of *Sn* competence, pigment accumulation takes place only in the presence of the *R-sc Sn-s* genotype and light (data not shown). The higher aleurone pigmentation of *Rsc Sn-s vs. R-sc sn* genotypes in darkness suggests that *Sn* substitutes for light, at least in part, in eliciting *R-sc* gene expression. (2) The amount of pigment conditioned by *Sn-s* is significantly higher in mesocotyls of *r Sn-s vs. R-r Sn-s* or *R-sc Sn-s* genotypes, suggesting a suppressive effect of *R* upon the pigmenting capacity of *Sn* (Table 6). These comparisons should be interpreted with caution, how-

TABLE 4

Instability of *Sn:bol3* vs. stability of *Sn:bol2*

Selection No.	*Sn* source	*n*	Parentals		Nonparentals		Segregation of	
			R Sn	*r-g sn*	*R sn*	*r-g Sn*	*R*	*Sn*
M19-4	*bol3*	3896	47.9 (12.0)	32.4	1.8	16.1 (0.6)	49.7	64.0
M25-2	*bol3*	1820	47.5 (14.3)	36.1	1.9	14.5 (0.5)	49.4	62.0
29-1	*bol3*	1831	46.0 (18.0)	31.2	1.0	21.7 (1.0)	47.1	67.8
	bol2	4734	48.9	49.3	0.9	0.8	49.8	49.8

Instability of *Sn:bol3* (three independent selections) compared with stability of *Sn:bol2* (one selection only) as determined in the progeny of *R-sc Sn/r-g sn* females mated to *r-g sn* males.

Progeny values are expressed as percentage. Those in brackets refer to *Sn-s* progeny seedlings while the accompanying values represent total *-s* and *-w* variants. *Sn:bol2* confers a homogeneous mesocotyl pigmentation intermediate between *-s* and *-w*.

TABLE 5

Effect of *R* constitution in *trans* on *Sn* instability

Progeny classes	*R* allele constitution			
	R-st	*R-g*	*R-r*	
R sn	42.3	38.6	0.0	
r Sn	48.1 (ND)[a]	49.8 (43.8)	22.1	(0.1)
R Sn	9.5 (1.4)	11.9 (1.5)	49.7	(22.0)
r sn	0.0	0.0	28.3	
Total No. seedlings	2639	4448	2677	

R-st sn, *R-g sn* or *R-r sn* plants heterozygous for *r Sn-s* were testcrossed by pollination with *r-g sn*.

Frequency ($\times 10^{-2}$) and verified constitution of chromosomes recovered in the testcrossed progeny of heterozygous females with one chromosome marked *r Sn-s* and the other one carrying *R-st sn*, *R-g sn* or *R-r sn*. The heterozygotes were obtained by pollinating homozygous *R-r*, *R-g* and *R-st* plants with pollen of an *r Sn-s* line. Chromosomal constitution of each progeny seedling was ascertained by progeny test. Figures in brackets refer to the frequency of progeny seedlings with strong mesocotyl pigmentation (*Sn-s*).

[a] Not determined.

ever, until the *Sn* constitution of nonparental *R Sn* chromosomes can be defined in more precise terms.

DISCUSSION

The various *Sn* isolates we analyzed are alleles obtained from different natural populations that differ as to when and how much anthocyanin is synthesized in specific tissues. They share the same response to *Pl* and show an identical pattern of nonreciprocal recombination. The uniformity of response to the unlinked *Pl* factor suggests functional identity of the various *Sn* sources. The meaning and the mechanism of nonreciprocal recombination still remains poorly understood. For the moment two different hypotheses are proposed (Figure 1). One assumes that the original *Sn* chromosome conferring an *r Sn* phenotype carries two separate *Sn* genes, one at the *R* locus and a second one two map units distal to *R*. Heterozygotes carrying a normal *R sn* chromosome and the *r Sn* homolog, should yield two kinds of recombinants following crossing over between *R* and the distally located *Sn*,

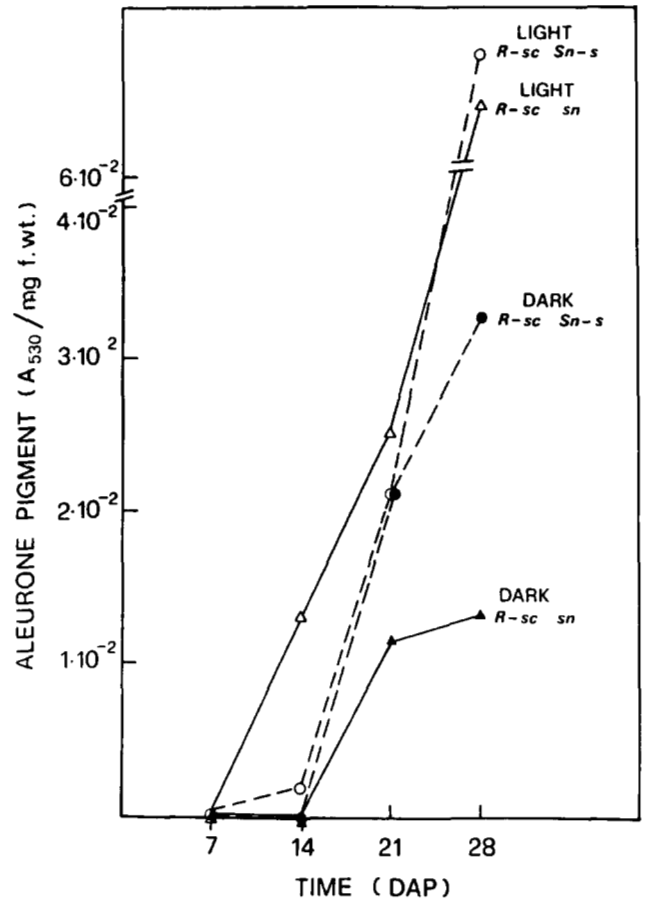

FIGURE 2.—Time course of anthocyanin accumulation in the aleurone of immature seeds removed at different days after pollination (DAP) from homozygous *R-sc Sn-s* and *R-sc sn* ears. Seeds were illuminated or kept in darkness for 96 hr before pigment extraction.

an *R Sn* class recognizable as nonparental progeny seedlings and an *r Sn sn* class indistinguishable from parental *r Sn Sn* chromosomes and thus undetectable. Alternatively, one could postulate that the original *Sn* chromosome carries a null *r* allele missing a viable function that is complemented by a function borne on the adjacent *Sn* gene region. A similar situation could arise if *Sn* represents a displaced *R* originating from transposition of *R* from its original position to an

TABLE 6

Frequency distribution and mean color scores of mesocotyls from heterozygous *Sn-s/sn* seedlings differing in their *R* constitution

Genotype	Mesocotyl color class 0	1	2	3	4	Mean score	Selected class	A_{530} at: 46 hr	96 hr
R-sc Sn-s / *r-g sn*			10	60		2.80	3	12.1	52.0
							2	2.6	16.0
R-r Sn-s / *r-g sn*	2	6	14	13		1.80	3	24.0	42.0
							1	1.2	11.4
r Sn-s / *r-g sn*		1	15		21	3.11	4	68.7	158.0
							2	4.4	31.0

Mesocotyl color class refers to the visual estimate of pigment content after 72 hr of continuous illumination. Mesocotyls were evaluated by matching them with a standard set of mesocotyls ranging from colorless (class 0) to strongly pigmented (class 4). Selected class refers to the visual scoring value of mesocotyls that were individually extracted to determine their pigment content spectrophotometrically. A_{530} is the mean absorbance value at 530 nm/g fresh weight as determined after 46 and 96 hr of continuous illumination. Each value represents the mean of six independent readings of single mesocotyl extracts.

adjacent site. The original chromosome could be designated *r*(−)*Sn*(+) to indicate this functional interdependence. Accordingly disruption of the *r-Sn* association by crossing over would lead to a *r*(−)*sn* chromosome impaired in some basic function and thus nontransmissibile, while the reciprocal *R-sc Sn*(+) class would be normally transmitted. Both hypotheses are consistent with the unusual pattern of recombination observed as well as with the resumption of normal recombination in heterozygous *R Sn/r sn* plants. The second hypothesis, however, would imply a functional relatedness of the two genes that finds some support in the reciprocal effects exerted by *R* and *Sn* upon pigment accumulation in aleurone and seedling tissues (Figure 2 and Table 6). On the other hand, the recent finding that insertion of a transposable element into either the *bz* or *R* locus affects intragenic recombination in various ways (DOONER and KERMICLE 1986) opens the way to other possible interpretations.

As emphasized in the RESULTS section, *Sn:bol3* differs from the other alleles in its instability. This is manifested as frequent changes of *Sn* from an *Sn-s* original allele to derivatives with significantly lower seedling pigmentation collectively referred to as *Sn-w*. It might well be that DNA modification could account for this instability since the frequent changes *Sn:bol3* undergoes from strong to weak variants, generally a unidirectional event, are specifically reversed by treatment with 5-azacytidine, a compound known to cause DNA hypomethylation (CEDAR 1988). Further experiments are required to confirm this possibility and to prove it at molecular level. However, this would not explain the appearance of weak derivatives of *Sn* on the nonparental chromosome in the progeny

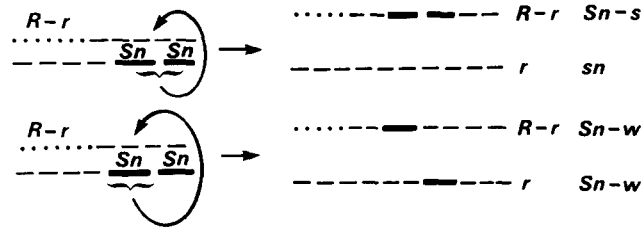

FIGURE 3.—Genetic constitution of nonparental chromosomes in the progeny of *R-r sn/r Sn-s* heterozygotes on the assumption that *Sn-s* is a compound structure consisting of two *Sn* elements capable of individual nonreplicative transposition.

of *Sn-s/sn* heterozygous plants or the effect of *R* locus constitution on the transfer of *Sn* information to its homolog.

It seems, rather, that *Sn* undergoes frequent passages from one chromosome to its homolog by transferring partial information. The chromosome constitution of individuals obtained by testcrossing *R-r sn/r Sn-s* plants suggests that loss of *Sn* from the *r* marked homolog is associated with "contamination" of the *R-r* bearing chromosome by *Sn* elements. Two kinds of gain-loss classes are found in the two chromosomes: (1) loss of *Sn* in the parental chromosome and gain on the other (*r sn* and *R-r Sn-s*); (2) a partial loss and gain (*r Sn-w* and *R-r Sn-w*). These two reciprocal kinds of classes are best interpreted as the result of complementary events.

To explain these data we suggest that *Sn* is composed of two units and that the strong and weak phenotypes recovered in the progeny of heterozygotes have two and one *Sn* units respectively. Both units can independently move from one chromosome to the other. In the progeny of a heterozygous *r Sn-s/R sn* genotype both donor (*r Sn-s*) and recipient (*R sn*) chromosomes can thus bear 0, 1, 2 doses of *Sn* as a result of these movements (Figure 3). Presence of *R-r* on the "recipient" chromosome would be instrumental, according to the model, in eliciting this passage since other *R* alleles do not show such a transactive effect.

The observation that the weak *Sn* expression expected in the presence of one Sn unit is represented by various levels of pigmentation could be explained by the occurrence of DNA modification events not detectable if two units are present. The high frequency of nonparental phenotypes could be explained by assuming that movements of the *Sn* units from one chromosome to the other are early events occurring in the germ cell line before meiosis. This assumption predicts the occurrence of sectors of seeds with nonparental seedling phenotype in the heterozygous parental ear. Preliminary data (G. GAVAZZI, unpublished results) based on ear mapping analysis fail to show the existence of sectors thus ruling out the involvement of premeiotic events in *Sn* transfer.

An alternative to the transfer explanation could be

the activation of a cryptic *Sn* gene by the active one carried on the other homolog. The finding that the rate of cryptic *Sn* activation is significantly increased if *R-r* is present on the same chromosome would imply the involvement of the *P* component of *R* in this activation process.

Once activated *Sn* would be capable of secondary activation of a silent *Sn* residing on an other homolog. Evidence for the activation or transfer hypotheses can be provided by a detailed molecular analysis of individual *R Sn* derivatives obtained by testcrossing *R sn/r Sn* parents to ascertain their *Sn* constitution. This analysis is presently under way.

Examples of transactive signals are well documented in plant literature, the most prominent being the phenomenon of paramutation (BRINK 1973) and, more recently, the finding in *Antirrhinum* of the activity of a specific allele of the *nivea* locus in *trans* to repress the level of transcript produced by its homolog (COEN and CARPENTER 1988).

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Communicating editor: B. BURR