Theor Appl Genet (1984) 69:71–77

Analysis of variants affecting the catalase developmental program in maize scutellum

J. M. Chandlee and J. G. Scandalios *
North Carolina State University, Department of Genetics, Raleigh, NC 27695, USA

Received March 20, 1984; Accepted April 4, 1984
Communicated by H. F. Linskens

Summary. The catalase of maize scutella is coded for by two loci, *Cat1* and *Cat2,* which are differentially expressed in this tissue during early seedling growth. Two variant lines have been previously identified in which the developmental program for the expression of the *Cat2* structural gene in the scutellum has been altered. Line R6-67 exhibits higher than normal levels of CAT-2 catalase in this tissue after four days of post-germinative growth. This phenotype is controlled by a temporal regulatory gene designated *Car1.* Line A16 exhibits a CAT-2 null phenotype. Further analysis of *Car1* verifies the initial indication that it is trans-acting and exhibits strict tissue (scutellum) specificity. A screen of other available inbred lines uncovered eight additional catalase high-activity lines. All eight lines exhibit significantly higher than normal levels of CAT-2 protein. Two of these lines have been shown to be regulated by *Car1* as in R6-67. Another line (A338) uncovered during the screen exhibits a null phenotype for CAT-2 protein and resembles A16. Catalase activity levels are low in the scutellum and no CAT-2 CRM (cross-reacting material) is present in the tissues of this line. Also, unlike most maize lines, CAT-2 cannot be induced in the leaf tissue of A338 upon exposure to light. Finally, a single line (A337), demonstrating a novel catalase developmental program, was identified.

Key words: *Zea mays* – Catalase – Gene expression – Regulatory genes

Introduction

The catalase ($H_2O_2 : H_2O_2$ oxidoreductase, E.C.1.11.1.6; CAT) gene-enzyme system of *Zea mays* L. has been under investigation for several years (for review, see Scandalios 1979) and provides a model system to investigate the factors controlling gene expression during development of a higher eukaryote. Maize catalase is coded for by three distinct structural genes, *Cat1, Cat2* and *Cat3,* each of which has been mapped to separate locations in the maize genome (Roupakias et al. 1980). The catalase genes exhibit a high degree of temporal and spatial specificity in their expression during development of the maize plant (Scandalios 1983). Maize scutellar catalase is coded for by two loci, *Cat1* and *Cat2,* which are differentially expressed in this tissue during early seedling growth (Scandalios 1979). In a typical line (e.g. –W64A) CAT-1 is expressed in the developing kernel, dry seed and during the early days post-imbibition. CAT-2 production becomes evident on days-2 and -3 post-imbibition. When both genes are expressed a five-banded zymogram pattern is observed due to the random association of the CAT-1 and CAT-2 subunits and the tetrameric structure of maize catalase (Quail and Scandalios 1971; Scandalios 1965). As development proceeds, CAT-1 levels decline whereas CAT-2 accumulates resulting in a shift of catalase gene expression. By day-10 post-imbibition only the *Cat2* gene is expressed. The expression of the two structural genes is regulated by several factors (Quail and Scandalios 1971; Scandalios 1974) one of which is differential turnover rates of the CAT-1 and CAT-2 gene products (Quail and Scandalios 1971).

The inbred maize line R6-67 exhibits significantly elevated levels of total scutellar catalase activity after the fourth day of post-germinative development (Scandalios et al. 1980), as compared to the characteristic catalase activity developmental program of most "typical' maize inbred lines examined (e.g. – W64A).

* To whom reprint requests should be addressed

Biochemical and genetic analysis has shown that an increase in synthesis of CAT-2 protein in R6-67 is controlled by a distinct temporal regulatory gene, designated *Car1*, which is located 37 map units from the *Cat2* structural gene (Scandalios et al. 1980). Two alleles are found at the *Car1* locus: $Car1^{L}$-responsible for low CAT-2 activity in the 10-day scutellum (e.g. – W64A) and $Car1^{H}$-responsible for high CAT-2 activity in the 10-day scutellum (e.g. – R6-67). The *Car1* regulatory gene exhibits additive inheritance and is time (acts after 4-days post-imbibition)-, and isozyme structural gene (*Cat2*)-specific in its action. Being distally located, *Car1* likely acts to modulate the expression of *Cat2* via a diffusible regulatory molecule.

The inbred maize line A16 exhibits a null phenotype for CAT-2 protein (Tsaftaris and Scandalios 1981). Total levels of scutellar catalase activity (primarily a reflection of CAT-2) are significantly reduced in A16 during early post-germinative development (Tsaftaris and Scandalios 1981). Electrophoretic and immunological studies (Tsaftaris and Scandalios 1981; Tsaftaris et al. 1983) confirm the lack of CAT-2 protein in the tissues of A16. In all maize lines examined to date CAT-2 activity can be induced in the leaf tissue upon exposure to light. However, this is not the case with A16 (Tsaftaris and Scandalios 1981; Tsaftaris et al. 1983). Genetic analysis indicates that the lack of CAT-2 protein is inherited as a single, recessive, Mendelian gene (Tsaftaris and Scandalios 1981). Whether the defect is controlled at the transcriptional or post-transcriptional level, or both, is not yet known. It is possible that the observed CAT-2 null phenotype is effected by a mutation at a closely linked regulatory locus or within the *Cat2* structural gene itself.

This report details our continued efforts to elucidate the mechanisms regulating catalase gene expression in the scutellum of maize. To accomplish this task it is necessary to better understand previously identified catalase regulatory variants as well as identify new variants for subsequent analysis. Together, these regulatory mutants can serve as an abundant source of material with which to analyze catalase gene expression. Analysis of enzyme expression provides a reasonable and promising approach toward understanding the regulation of gene expression in eukaryotes. Enzymes provide a sensitive probe to study the developmental program of expression of the encoding genes and can aid in the effort to elucidate, at least at the post-translational level, the underlying mechanisms controlling differential gene expression. The information gained at this level of gene expression will eventually prove useful in subsequent attempts to more precisely analyze the regulation of catalase gene expression at the transcriptional and post-transcriptional level. Information is presented here on the further characterization of the *Car1* temporal regulatory gene with regard to its mode of action. Other catalase high activity lines are described. A second *Cat2* catalase null line is described, and a line which exhibits a novel catalase developmental program is discussed.

Materials and methods

Plant material

The highly inbred maize lines W64A, R6-67 and A16 which were used in these studies, as well as approximately 150 inbred lines used in the screen for altered catalase expression are maintained by our laboratory. An additional 130 lines obtained from R. H. Moll (N. C. State University) were also screened. The second set of lines were not highly inbred but were found, in most cases, to be homozygous at the *Cat1* and *Cat2* loci, the genes of interest in this study.

Growth of seedlings

Seeds were surface-sterilized for 10 min in a 1% sodium hypochlorite solution (20% commercial clorox) and soaked in distilled water for 24 h. The seeds were then placed in plastic containers on several layers of germination paper moistened with distilled water. Germination and subsequent growth were effected at 23 °C in the dark unless otherwise stated.

Sample preparation

Isolated scutella of post-germinative seedlings, as well as root and coleoptile tissue, were ground with sand in glycylglycine buffer (25 mM, pH 7.4) using a pre-chilled mortar and pestle. The homogenate was centrifuged in an Eppendorf microfuge for 3 min and the supernatant was used for either starch gel electrophoresis, spectrophotometric assays, rocket immunoelectrophoresis or immunodiffusion experiments. Dark- or light-grown leaf tissue was ground in HEPES buffer (50 mM, pH 7.5) including 1mM DTT and treated as above.

Starch gel electrophoresis

Starch gel electrophoresis using the tris citrate (1.0 M, pH 7.0) buffer system was performed as previously described (Scandalios 1969). Catalase was stained by first incubating a gel slice in 0.01% H_2O_2 for 20 min after which the slice was rinsed and stained with a solution consisting of 1% $FeCl_3$ and 1% $K_3Fe(CN)_6$.

Enzyme assays and protein determination

Catalase (Beers and Sizer 1952), malate dehydrogenase (Yang and Scandalios 1975), alcohol dehydrogenase (Felder et al. 1973) and superoxide dismutase (Beauchamp and Fridovich 1971) were assayed spectrophotometrically as described. Protein concentration was determined (Lowry et al. 1951) using bovine serum albumin as a standard.

Immunoelectrophoresis and immunodiffusion experiments

The technique of rocket immunoelectrophoresis was performed as previously described (Laurell 1966) with minor modifications. Fifty milliliters of 1% agarose (Bio-Rad, Low M_r) in tris-HCl buffer (50 mM, pH 8.6) was boiled and then cooled to 57 °C. At this point an appropriate amount of CAT-2 antibody, prepared as previously described (Chandlee et al. 1983), was added to the solution which was then poured onto a 23 × 12.5 cm glass plate. After cooling, samples of identical

protein content were added to 1 mm diameter wells. Electrophoresis was conducted at 4 °C with 60 V for 48 h. The gel was then pressed and washed as described (Laurell 1966) except that the electrophoresis buffer was used and the whole procedure was performed at 4 °C to avoid enzyme inactivation. The gel was then stained for catalase activity as described above. The area under each rocket was estimated by multiplying the height of each rocket in mm by its width at half-height (Axelson et al. 1973). Ouchterlony double immunodiffusion plates were prepared as described (Ouchterlony 1968) and immunodiffusion was conducted at 4 °C in humidified chambers for 3–4 days.

Results

Mode of action of the Car1 temporal regulatory gene

To determine if the *Car1* regulatory gene affects catalase expression in tissues other than the scutellum, catalase assays were performed on extracts of coleoptile, root and dark- and light-grown leaf tissue from W64A (low catalase activity line), R6-67 (high catalase activity line regulated by *Car1*) and D10 (high catalase activity line possibly regulated by *Car1*; see below). The results (Fig. 1) indicate that *Car1* exerts a tissue-specific effect in the scutellum of maize. Of the tissues assayed, only the scutellum and light-grown leaf tissue express the CAT-2 isozyme. The coleoptile and dark-grown (etiolated) leaf tissue express CAT-1 and CAT-3 while root tissue only expresses CAT-1. Therefore, *Car1* is specific both to the catalase structural gene it affects and the tissue in which it exerts its effect.

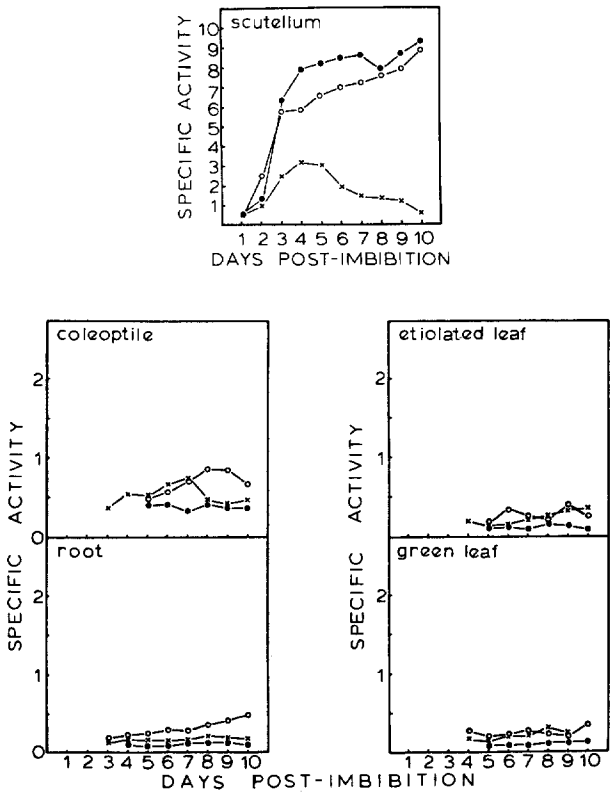

Fig. 1. The catalase specific activity (ΔA/min/mg protein) developmental program in various tissues of maize from W64A (× --- ×), R6-67 (○ ---○) and D10 (● ---●) during the first 10-days post-imbibition. The following isozymes are expressed in each tissue: *scutellum* CAT-1 and CAT-2 (see text for a discussion on the shift from CAT-1 to CAT-2 expression); *coleoptile* CAT-1, CAT-3; *root* CAT-1; *etiolated leaf* CAT-1, CAT-3; *green leaf* CAT-2, CAT-3. Each point represents the mean of three independent experiments

In order to verify previous recombination data (Scandalios et al. 1980) suggesting that *Car1* is a trans-acting element, F_1 hybrid seed were produced which were heterozygous at both the *Cat2* and *Car1* loci. Extracts were made from 10-day post-imbibition scutella (only CAT-2 is expressed) of the F_1 seed and subjected to starch gel electrophoresis. After staining for catalase activity, the gel patterns were scanned densitometrically (Fig. 2) using a "Zieneh Soft Laser Scanning Densitometer" (Biomed Instruments, Model SL-504). The control F_1 hybrid seed (heterozygous for *Cat2* and homozygous for *Car1*) exhibits a symmetrical distribution for the five catalase bands. This pattern is also observed in the test F_1 hybrid seed (heterozygous for both *Cat2* and *Car1*) indicating that *Car1* is trans-acting and that it equally affects both copies of the *Cat2* structural gene.

Screen of available inbred lines for altered catalase expression

One-day-old and 10-day-old scutella isolated from approximately 150 highly inbred lines were assayed for catalase activity. The 1-day-old scutella of all lines exhibited typically low levels of catalase activity. Of the 10-day-old scutella, most lines exhibited low levels of activity and resembled the "typical" line W64A ($Car1^L$). However, several lines exhibited high levels of activity similar to R6-67 ($Car1^H$) while others demonstrated intermediate levels of activity resembling *Car1* heterozygotes ($Car1^H/Car1^L$) (Table 1). Those lines having high catalase activity were analyzed further. All of them exhibited a catalase developmental program similar to that seen in R6-67 (Fig. 1). These lines were examined with rocket immunoelectrophoresis using CAT-2 antiserum and all showed elevated levels of CAT-2 protein in the 10-day-old scutellum, much like R6-67 (Table 2). The developmental pattern of several other enzymes were examined in these lines also, and no differences were found for malate dehydrogenase, alcohol dehydrogenase or superoxide dismutase.

Some of the high activity lines were analyzed to determine if they are regulated by *Car1*, as is R6-67. F_1 and F_2 generation seed were produced from various crosses between R6-67, D10 and 59. The 10-day-old scutella were then assayed for catalase activity. If

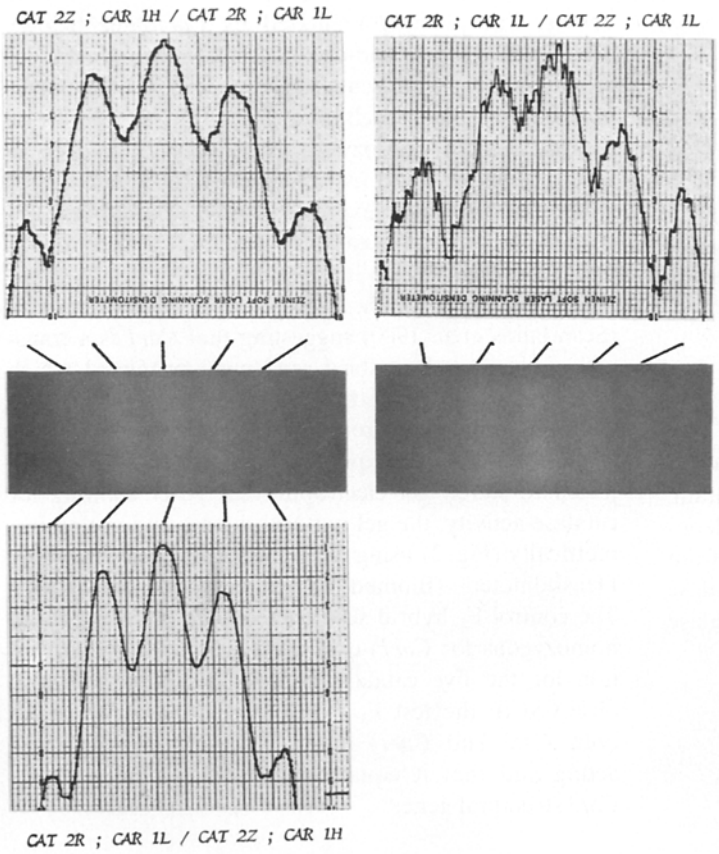

Fig. 2. Densitometric tracings of the catalase zymogram pattern produced from 10-day scutellar extracts (only *CAT-2* is expressed) of the following F_1 hybrids: R6-67×373 (*Cat2Z; Car1*^{H}/*Cat2R; Car1*^{L}); 373×R6-67 (*Cat2R; Car1*^{L}/*Cat2Z; Car1*^{H}); and the control 128×133 (*Cat2R; Car1*^{L}/*Cat2Z; Car1*^{L}). The pattern of the densitometric scan is symmetrical and not skewed toward either *CAT-2* homotetramer (*CAT-2R* or *CAT-2Z*), even when *Car1* is heterozygous, indicating that *Car1* is transacting

Table 1. Distribution of maize inbred lines (total = 147) with respect to 10 day scutellar catalase activity levels

High activity lines (> 8 units/mg protein)	Low activity lines (< 4 units/mg protein)	Intermediate activity lines (4–8 units/mg protein)
9 (6.12%)	120 (81.63%)	17 (11.56%)

Table 2. Catalase-2 rocket areas for various maize inbred lines demonstrating high scutellar catalase activity after 10 days post-imbibition

Inbred line	Cat-2 protein Area (mm²)/mg protein	
W64A	933.54± 79.51	(low activity control)
R6-67	2,180.29± 144.64	(high activity control)
C10	2,636.91± 164.66	
A165	2,299.43± 140.13	
59	2,206.90± 185.88	
332	2,077.34± 80.33	
W_7	2,277.96± 116.31	
BM107	1,813.00± 201.87	
378	2,431.01± 360.51	
D10	1,913.41± 76.80	

D10 and 59 are allelic to R6-67 at the *Car1* locus, then both the F_1 and F_2 generation seed should have the same level of catalase activity as R6-67, as well as demonstrate similar standard deviations to the parent lines. If D10 and 59 are being controlled by a second regulatory gene, independent of *Car1*, then the F_1 seed activity and standard deviations should resemble either parent, but the F_2 seed should have standard deviations greater than either parent as expected for any segregating F_2 population. This is not the case (Table 3). Therefore, 59, D10 and R6-67 are allelic at the *Car1* locus. At present it is not known whether the remaining six high activity lines are controlled by *Car1* or a second regulatory gene. The allelism tests are continuing.

During the course of screening for high activity variants, one line (A337) was found which exhibits a novel catalase specific activity developmental program in the scutellum (Fig. 3); this pattern is highly reproducible. This line is currently being analyzed to determine whether or not the increased level of catalase is genetically controlled.

Identification of a second CAT-2 protein null variant

During the process of screening a second set of maize lines (less inbred than the above set) for altered

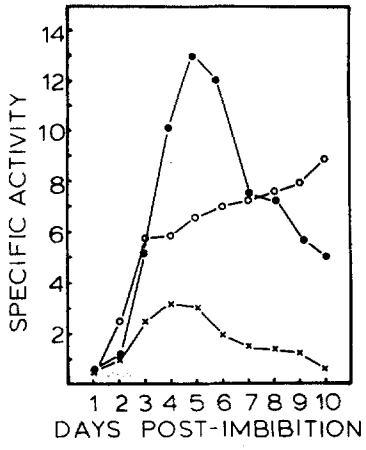

Fig. 3. The catalase specific activity (ΔA/min/mg protein) developmental program in the scutellum of W64A (× --- ×), R6-67 (○ ---○) and A337 (● ---●) during the first 10-days post-imbibition. Notice the novel pattern in the line A337. Each point represents the mean of three independent experiments

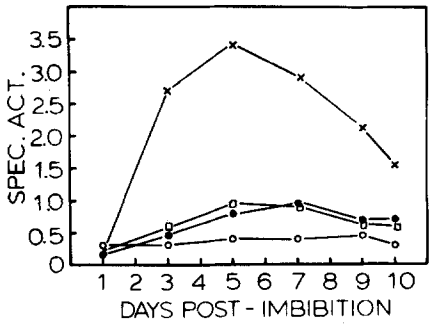

Fig. 4. The catalase specific activity (ΔA/min/mg protein) developmental program in the scutellum of W64A (× --- ×), A16 (○ ---○), A338 (● ---●) and the F_1 hybrid A16×A338 (□ ---□) during the first 10-days post-imbibition. Each point represents the mean of three independent experiments. The minimal activity observed in A16 is known to be due to residual amounts of CAT-1

catalase expression, one line (A338) was found with unusually low levels of catalase activity. The catalase specific activity developmental program was found to be similar to A16 (Fig. 4) a previously identified CAT-2 protein null variant (Tsaftaris and Scandalios 1981). Zymogram analysis of scutellar extracts from A338 (Fig. 5 A) indicated a lack of CAT-2 activity. Immunological studies (Fig. 5 B) indicated a lack of CAT-2 CRM (cross-reacting material) in the tissues of this line. Also CAT-2 activity cannot be induced in the leaf tissue of A338 upon exposure to light (Fig. 6). At present it is not known whether the defect in *Cat2* gene expression is similar in A16 and A338 but, the F_1 hybrid (A16×A338) does not show complementation. Catalase activity levels in the scutellum of the F_1 hybrid remain low (Fig. 4) and no CAT-2 activity is detectable on zymograms.

Table 3. Means and standard deviations of catalase specific activity in 10-day scutella in samples of parental inbreds, F_1 and F_2 generations

Line	Catalase activity		
	N	Mean	SD
Parents			
R6-67	50	8.95	1.80
D10	48	8.07	1.64
59	50	9.30	1.93
F_1 Crosses			
D10×R6-67	89	8.05	1.71
R6-67×D10	78	8.26	1.68
F_2 Crosses			
(D10×R6-67)⊗	106	8.11	1.88
(R6-67×D10)⊗	104	8.59	1.83
(59×D10)⊗	150	9.33	1.94
(D10×59)⊗	150	8.26	1.78

Catalase activity = Δ abs/min/mg protein

Discussion

The catalase gene-enzyme system of maize provides an excellent model with which to analyze gene expression in a higher eukaryote since the genes are highly regulated during the development of the maize plant (Scandalios 1979; Scandalios 1983). The developmental program for maize scutellar catalase activity results from the differential expression of two *Cat* structural genes, *Cat1* and *Cat2*. The expression of these two genes is regulated by several factors including an endogenous maize catalase inhibitor, hormones (ABA) and differential turnover rates (Quail and Scandalios 1971; Scandalios 1974, 1979). Also, maize scutellar catalase activity is influenced by two temporal regulatory genes. The first, *Car1*, specifically affects *Cat2* gene expression by controlling the rates of CAT-2 protein synthesis (Scandalios et al. 1980). The second regulatory gene, *Car2*, specifically affects expression of the *Cat1* gene by influencing the overall levels of CAT-1 protein synthesis (manuscript in preparation). It is apparent that the final realization of the catalase developmental program in maize scutella is attained through extensive regulation (both genetic and epigenetic) of the expression of the *Cat1* and *Cat2* structural genes. Eventual understanding of the precise molecular mechanisms regulating this differential gene expression will be achieved through the analysis of available regulatory variants. The purpose of this study

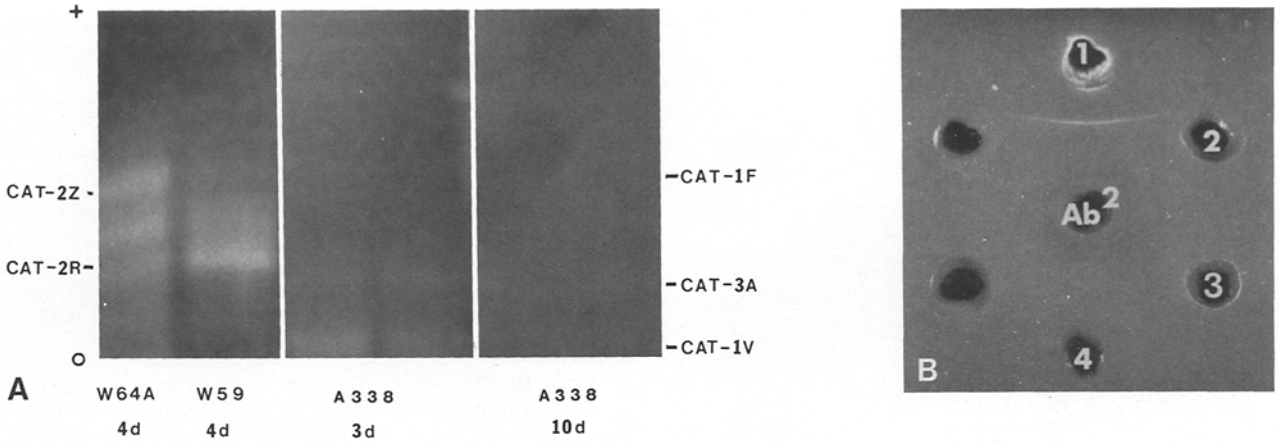

Fig. 5. **A** Zymogram analysis of catalase gene expression in the scutellum of *A338* during early sporophytic growth. The first two lanes represent 4-day (4d) post-imbibition scutellar extracts of *W64A* and *W59* and are used as markers. 3-day (3d) post-imbibition scutellar extracts of *A338* exhibit faint traces of *CAT-1* and *CAT-3* activity. 10-day (10d) postimbition scutellar extracts of *A338* show no trace of the normally expected *CAT-2* activity. Migration is anodal; *O* point of sample insertion. **B** Ouchterlony immunodiffusion plates showing the lack of *CAT-2 CRM* (cross-reacting material) in various tissues of *A338*. *CAT-2* antiserum was placed in the center well. The outer wells contain: 1–4-day post-imbibition *W64A* scutellar extract; 2–3-day post-imbibition *A338* scutellar extract; 3–10-day post-imbibition *A338* scutellar extract; 4–10-day post-imbibition *A338* green leaf extract (in light for 3 days)

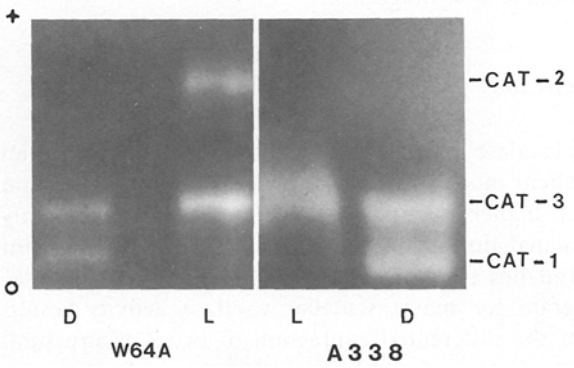

Fig. 6. Zymogram of catalase patterns in the leaf extracts of *W64A* and *A338* grown in dark (*D*) or light (*L*). Notice the lack of induction of *Cat2* gene expression in the light grown leaf of *A338*. Migration is anodal; *O* point of sample insertion

was to increase our understanding of the previously characterized catalase genetic regulatory variants and also to search for additional variants to add to the growing collection of maize lines exhibiting altered catalase expression in the scutellum. Ultimately, these lines will prove useful in our attempts to more precisely analyze the regulation of catalase gene expression at the transcriptional and post-transcriptional level.

A number of regulatory genes that are either proximal or distal to the structural locus they control and that determine the level of expression of certain enzymatic proteins have previously been identified (Chandlee and Scandalios 1984). Such regulatory genes are found not only in maize (Lai and Scandalios 1980; Scandalios et al. 1980), but in other plants (Scandalios and Baum 1982) and animals (Paigen 1979) as well. Temporal regulatory genes (Paigen 1979) determine the developmental program for the concentration changes that each protein undergoes during cellular differentiation. Inherent in this definition is the implication of both tissue- and temporal-specificity in the action of these genes. Previous data (Scandalios et al. 1980) coupled with the data presented in this report confirm the tissue- and temporal-specific action of the *Car1* gene. Most distant regulatory genes are trans-acting (Chandlee and Scandalios 1984). In other words, both copies of the structural gene being regulated are equally affected by the action of the regulatory gene. The evidence presented suggesting that *Car1* acts in trans is not totally unexpected since it is located distal to the *Cat2* gene and presumably functions via a diffusible regulatory molecule. However, it is unusual that a trans-acting regulatory gene would exhibit additive inheritance (Scandalios et al. 1980). Therefore, some novel regulatory mechanism must exist in eukaryotes since *Car1* cannot be acting through the simple repressor/activator (i.e. – on/off switch) mechanism found in prokaryotes.

The analysis of eight additional catalase high activity lines, other than R6-67, suggests the possibility that *Cat2* may be regulated by more than one regulatory gene. While D10 and 59 appear to be allelic to *Car1* of R6-67, this has yet to be proven for the remaining six lines and leaves open the possibility of a

second regulatory gene affecting *Cat2* gene expression. This idea is further supported by the identification of a maize line (A337) which exhibits a new and novel pattern of catalase expression in the scutellum. At present, however, it is unclear as to whether *Cat1* or *Cat2* gene expression is altered in this line and whether this variation is genetically controlled. Analysis is currently underway.

The discovery of a second *Cat2* null variant may prove interesting since it is possible that A338 and A16 represent different defects in the steps leading to eventual expression of the *Cat2* gene. Molecular analysis, currently underway, of the *Cat2* gene structure in each line may uncover sequences necessary for the proper expression of the gene. Also, it is possible that A338 may represent a mutation in a regulatory gene controlling *Cat2* expression. This has been shown not to be the case in A16 (Tsaftaris and Scandalios 1981). Further analysis of the defect in A338 is currently underway.

In summary, as more maize lines are identified and characterized which demonstrate altered patterns of catalase expression, it will be possible to utilize these numerous variants in studies designed to probe the precise molecular mechanisms controlling the expression of the maize catalase genes. The greater the number of variants identified, the better the likelihood of gaining a true understanding of the regulation of catalase gene expression in maize.

Acknowledgements. The excellent technical assistance of Stephanie Rusza is gratefully acknowledged. This work was supported by Research Grant GM 22733 from the National Institutes of Health to J.G.S. This is Paper No. 9236 of the Journal Series from the North Carolina Agricultural Research Service, Raleigh, NC.

References

Axelson NH, Kroll V, Weeke B (1973) A manual of quantitative immunoelectrophoresis: methods and application. Universitets-Forlaget, Oslo

Beauchamp CO, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287

Beers PF, Sizer IW (1952) A spectrophotometric assay for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 195:133–140

Chandlee JM, Scandalios JG (1984) Regulatory genes of higher eukaryotes. Isozyme Bull 17:12–20

Chandlee JM, Tsaftaris AS, Scandalios JG (1983) Purification and partial characterization of three genetically defined catalases of maize. Plant Sci Lett 29:117–131

Felder MR, Scandalios JG, Liu EH (1973) Purification and partial characterization of two genetically defined alcohol dehydrogenase isozymes in maize. Biochim Biophys Acta 318:149–159

Lai Y-K, Scandalios JG (1980) Genetic determination of the developmental program for maize scutellar alcohol dehydrogenase: involvement of a recessive, trans-acting, temporal regulatory gene. Dev Genet 1:311–324

Laurell C (1966) Quantitative estimation of proteins by electrophoresis in agarose gels containing antibodies. Anal Biochem 15:45–52

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Ouchterlony O (1968) Handbook of immunodiffusion and immunoelectrophoresis. Ann Arbor Science Publ, Ann Arbor Mich

Paigen K (1979) Acid hydrolases as models of genetic control. Annu Rev Genet 13:417–466

Quail PH, Scandalios JG (1971) Turnover of genetically defined catalase isozymes in maize. Proc Natl Acad Sci USA 68:1402–1406

Roupakias D, McMillin DE, Scandalios JG (1980) Chromosomal location of the catalase structural genes in *Zea mays* using B-A translocations. Theor Appl Genet 58:211–218

Scandalios JG (1965) Subunit dissociation and reassociation of catalase isozymes. Proc Natl Acad Sci USA 53:1035–1040

Scandalios JG (1969) Genetic control of multiple molecular forms of enzymes in plants: a review. Biochem Genet 3:37–79

Scandalios JG (1974) Subcellular localization of catalase variants coded by the Ct_1 and Ct_2 genes during development. J Hered 65:28–32

Scandalios JG (1979) Control of gene expression and enzyme differentiation. In: Scandalios JG (ed) Physiological genetics. Academic Press, London New York, pp 63–107

Scandalios JG (1983) Molecular varieties of isozymes and their role in studies of gene regulation and expression during eukaryote development. In: Rattazzi MC, Scandalios JG, Whitt GS (eds) Isozymes: current topics in biological and medical research, vol 9. Alan R Liss, New York, pp 1–31

Scandalios JG, Baum JA (1982) Regulatory gene variation in higher plants. Adv Genet 21:347–370

Scandalios JG, Chang D-Y, McMillin DE, Tsaftaris AS , Moll RH (1980) Genetic regulation of the catalase developmental program in maize scutellum: identification of a temporal regulatory gene. Proc Natl Acad Sci USA 77: 5360–5364

Tsaftaris AS, Bosabalidis AM, Scandalios JG (1983) Cell-type-specific gene expression and acatalasemic peroxisomes in a null *Cat2* catalase mutant of maize. Proc Natl Acad Sci USA 80:4455–4459

Tsaftaris AS, Scandalios JG (1981) Genetic and biochemical characterization of a *Cat2* catalase null mutant. Mol Gen Genet 181:158–163

Yang NS, Scandalios JG (1975) De novo synthesis and developmental control of the multiple gene-controlled malate dehydrogenase isozymes in maize scutella. Biochim Biophys Acta 384:293–306