BIOCHEMICAL ASPECTS OF A GENETICALLY DETERMINED VARIEGATION IN ARABIDOPSIS[1,2]

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DIFFERENTIATION and development are controlled by genes (cf. HADORN 1961). These processes require selective synthesis or catabolism of enzymes or enzyme regulators. Little is known as to how the genes determine the selectivity and timing required for development (cf. URSPRUNG 1965). Several models of differentiation have been suggested on the basis of microbial experiments. Evidence that differentiation in higher forms is mediated through these models is not yet available. However, there are certain similarities in the control systems of phylogenetically different forms (MCCLINTOCK 1965). In Arabidopsis several mutants are known which lend themselves to the analysis of the mechanisms of development (RÉDEI 1962; HIRONO and RÉDEI 1966a,b). A variegation mutant, *im* appears specially suited for the study of differentiation. In tissues of the homozygous mutant, which are variegated with normal and white plastids, the level of an acid ribonuclease is elevated and inorganic phosphate accumulates, suggesting that abnormal RNA metabolism is related to the abnormal chloroplast formation.

MATERIALS AND METHODS

Mutant *im* was obtained by X-ray treatment (10,000r) of 24-hr presoaked seed of the Columbia wild type of *Arabidopsis thaliana* (Figure 1). This recessive mutant gene has a normal Mendelian transmission, and close linkage to markers vc_2 and *im* has been established. In homozygous condition *im* produces variegation: chloroplast differentiation cannot be completed in certain cells of the mutant, yet in others normal chloroplast development apparently takes place. The cells with imperfect plastids remain white; neither chlorophylls nor carotenoids are synthesized. All plastids within a single cell are either completely normal or abnormal (Figure 2). In the white cells all of the plastids are arrested in the same developmental stage. The cell phenotype is not fixed at the time of cell replication since the same seed sample may germinate with either all green or all white cotyledons depending upon the intensity or quality of light exposed to after imbibition. The expression is irreversible, however, after the completion of differentiation. The two types of cells are genetically identical, since the descendants of both kinds of cells may become either white or green. This peculiar behavior of the gene results in a characteristic nonrandom pattern of green and white cells with sharply demarcated areas in the various tissues. Some leaves carry large continuous sectors, while other display frequent transitions from green to white cell colonies closely following the pattern of differentiation. Sometimes a large number of cells of common descent undergo an apparently synchronized transition from one phenotypic

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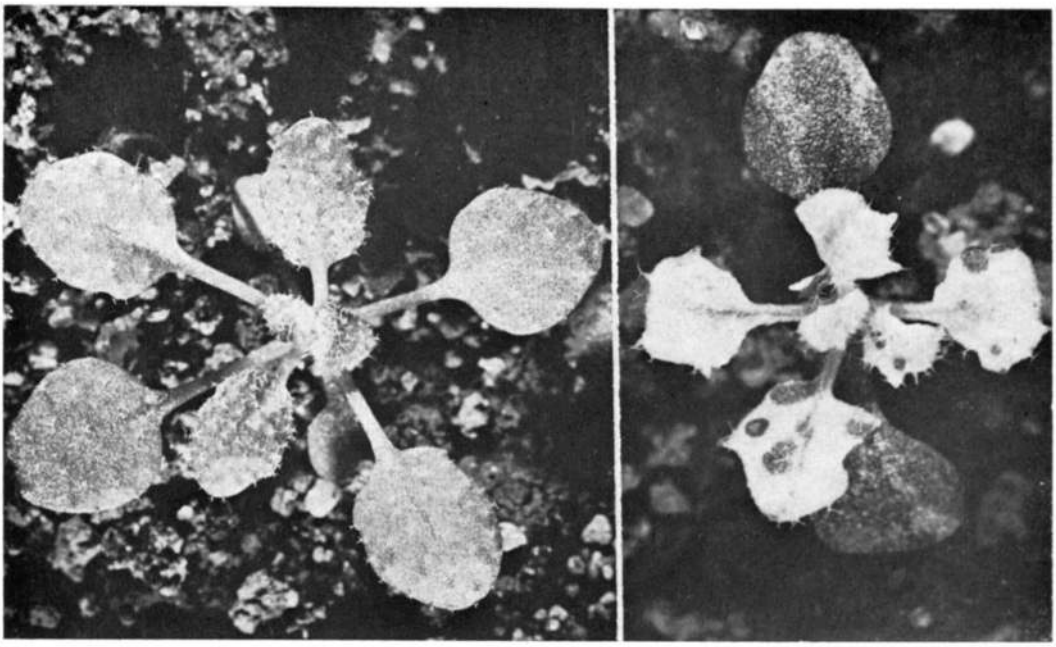

FIGURE 1.—Left, wild type of Arabidopsis. Right, mutant *im* grown in soil in the greenhouse under continuous illumination. Note the variegation in color on the mutant plant.

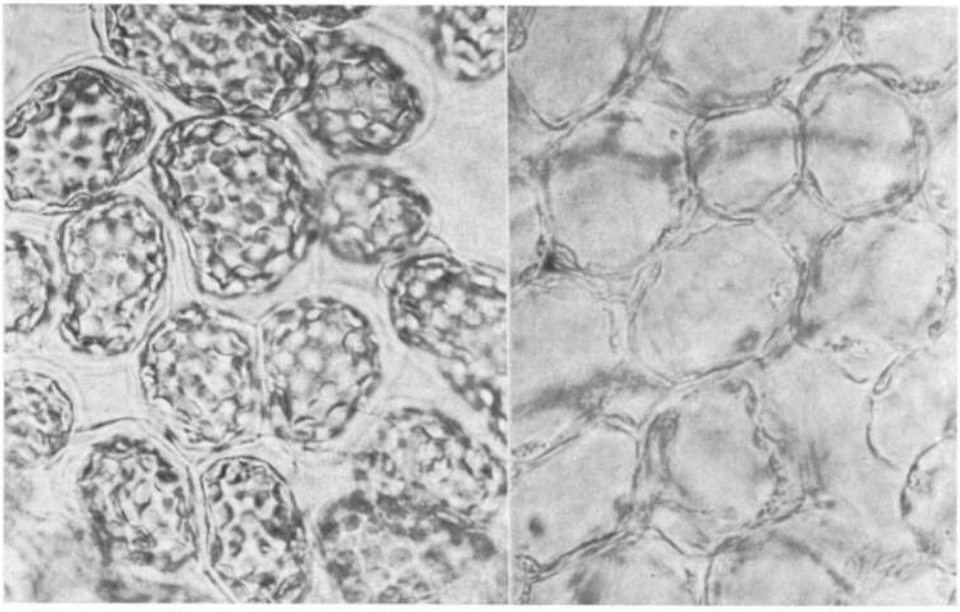

FIGURE 2.—Microscopic photograph of green (left) and white cells (right) of mutant *im*. In the green cells the chloroplasts are not distinguishable from those of the wild type; in the white cells only small plastids are found which are devoid of lamellar structure. Intact leaves were evacuated in water:glycerol:formalin (10:2:0.5) mixture and directly mounted on slides for examination.

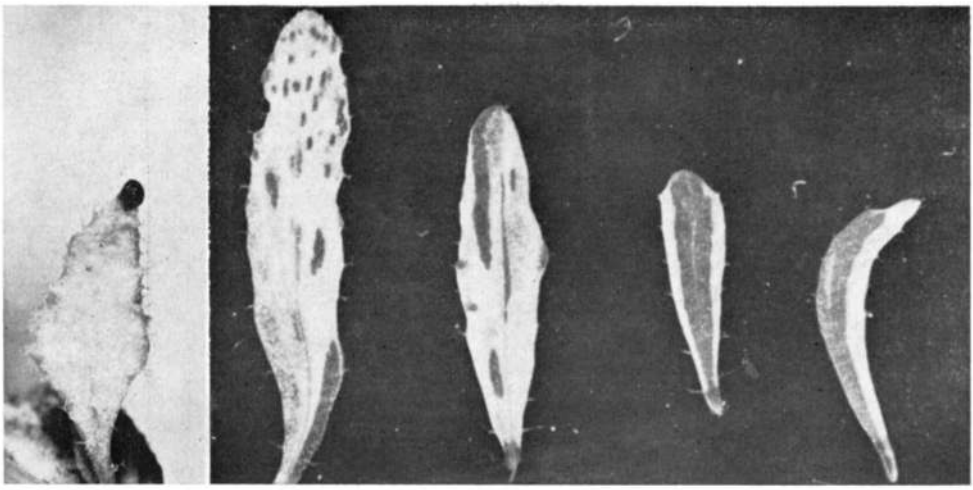

FIG. 3.—Stem leaves of the mutant. Many of the sectors are narrower at the base. The meristematic region in the leaves of Arabidopsis is located at the base, and therefore the shape of the sectors indicates the pattern of differentiation. These leaves contain only two or three layers of parenchyma cells, thus cell lineages can be traced without any difficulty. The leaf at left did not contain any green cells at the base, yet there is a green spot of considerable size at the tip. In the second and third leaves several groups of cells simultaneously changed back and forth to the production of green or white mitotic progenies. The greyish areas represent green tissues on the ventral side of the leaf blade which are covered by white cells of one or two layers. The pattern of leaves 4 and 5 is the result of a rather faithful replication of the cells of the original four-cell leaf initial.

state of the variegation to the other (Figure 3). In extreme cases single green cells or cell lineages may be found in a white cell environment.

For most of the biochemical studies, the double mutant *im gi*^{2} was used. The *gi*^{2} factor convays little sensitivity to photoperiodic treatment. Even under continuous illumination, which was desirable for producing an extreme mutant phenotype, vigorous vegetative growth was ensured because of the late flowering of the plants (cf. RÉDEI 1962). The *im*$^+$ *gi*^{2} *xv gl*^{2} genotype was selected as "wild type" control for most of the experiments. Since homozygous *im* is very low in pigment content, development is at a considerably slower rate than the *im*$^+$ type. This developmental difference was eliminated by employing the yellow-green gene *xv* in conjunction with *im*$^+$. Marker *xv* is closely linked to *gi* (about 2 units apart), and both are independent of *im*. The marker *gl*^{2} (hairless; of no importance in this system) is also independent of all other markers.

Plants for biochemical analysis were raised in the greenhouse. The pots were supplied with distilled water from below to prevent contamination of the leaves with mud or water salts and to assure vigorous growth. Occasionally test tube grown material was also used (cf. RÉDEI 1965).

Phosphorus was analyzed by the ammonium vanadate-ammonium molybdate technique of GERICKE and KURMIES (1952) from the same type of extracts which were used for the protein assay. Calcium and magnesium were estimated from dry ashed material according to the techniques of SENDROY and SIMONSEN *et al.* respectively as given by BALLENTINE and BURFORD (1957). Protein content was determined in the various buffer extracts according to the method of LOWRY, ROSEBROUGH, FARR and RANDALL (1951). The crude enzyme extract was obtained by homogenization of fresh or lyophilized plants in the appropriate buffers followed by centrifugation at 10,000 rpm at room temperature. This crude extract was practically colorless. The amount of inorganic phosphorus liberated in the incubation mixture was used as a measure of activity of the different

phosphatases. The less sensitive phenopthalein phosphate technique of HIGGINS and TALALAY (1945) was also employed as given by BERGMEYER (1963). Phosphodiesterase was assayed principally according to RAZZEL and KHORANA (1959). Ribonuclease activity was estimated by a slight modification of the technique of DICKMAN, AROSKAR and KROPF (1956). All enzymes were compared on the basis of equal amounts of protein. The data reported here were obtained from crude extracts. Enzyme extracts purified approximately 15 times were also tested with essentially the same results.

Subcellular fractions were prepared from fresh tissues disintegrated with a high speed Virtis homogenizer containing 0.5 M sucrose in pH 5.2 phosphate buffer (0.05 M). The chopping was stopped when almost all cells were broken, and the great majority of the chloroplasts still appeared intact as ascertained by microscopic examination. The homogenate was filtered through cheesecloth and then separated into fractions with a Spinco Model L analytical centrifuge. The fractions were freeze-dried, defatted by acetone, dried, ground in 0.5 M KCl, and then centrifuged at 10,000 rpm. The supernatants were dialyzed for 24 hours against cold distilled water and were preserved by lyophilization until used. Commercial yeast RNA, purified (FRISCH-NIGGEMEYER and REDDI 1959) from acid soluble nucleotides, was used as the substrate for ribonuclease assay. Other reagents were obtained either from Sigma (St. Louis, Mo.) or from Calbiochem (Los Angeles, California).

RESULTS

General characterization of the mutant: The response of *im/im* to temperature, to the quality and intensity of visible light, to X-rays, to the metabolites of the cysteine pathway and to 6-azauracil has already been mentioned (RÉDEI 1963a, 1965a). The genetic mechanism involved has also been discussed (RÉDEI 1963b). The activity of the *im* locus will be further characterized below.

A surprising observation was that high doses of X rays (10,000r) normalized to a small but definite extent the mutant phenotype. The number and size of the green sectors increased after irradiation (RÉDEI 1963b, 1967a). Similarly, the antimetabolite 6-azauracil more or less restored normal plastid differentiation when the mutant was grown for at least 3 or 4 weeks on a medium containing the analog (RÉDEI 1965a, 1967b). Feeding cysteine also made the plants greener, but its effect was less than that of 6-azauracil. The mutant did not respond much to the nutrients of a complete medium. These observations indicated that the mutant phenotype is not the consequence of the lack of synthesis of an essential nutrient; instead, the overactivity of an enzyme appears to be responsible for the disturbance.

Chemical analyses: From the knowledge that 6-azauracil interfered with the decarboxylation of orotidylic acid (HANDSCHUMACHER 1960), one might expect that the cells produce free inorganic phosphorus in the process of disposing of the accumulated orotidylic acid. Indeed, a simple chemical analysis revealed that the readily soluble phosphorus accumulates in the mutant. This difference is especially obvious in the white tissues (Table 1, Experiments 1, 2). The two samplings shown in Table 1 (Experiments 1, 2) were made on slightly different material and with different solvents. This data still proved consistent. In other experiments, the almost completely white *im/im* mutants accumulated even larger amounts of phosphorus. The analyses of several different samples gave variable results depending on the extent of variegation of the plants. The mutants displayed, however, more phosphorus than the wild type in every case.

TABLE 1

Comparison of genotypes im+/im+ *and* im/im

Experiment No.	Property compared	*im*+ Mean ± SE	*im* Mean ± SE	*im* as percent of *im*+
	Inorganic phosphorus			
1	in pH 7.5 Tris extract	.19 ± .01	.27 ± .01	141
2	in pH 6.6 citrate extract	.28 ± .04*	.54 ± .02†	194*
3	Calcium	.329 ± .011	.312 ± .006	95
4	Magnesium	.226 ± .003	.227 ± .004	100
5	Protein	11.3 ± .5	10.8 ± .7	96
6	Phosphomonoesterase‡	.192 ± .006	.201 ± .002	105
7	Phosphodiesterase§	.615 ± .010	.632 ± .033	103
	Phosphorus, μg liberated by crude enzyme extracts from‖			
8	2′-adenosine monophosphate	3.8 ± .1	3.2 ± .1	84
9	5′-adenosine monophosphate	7.4 ± .0	7.5 ± .1	101
10	3′-adenosine monophosphate	28.8 ± .5	23.6 ± .7	82

* The Experiment 2 value in the *im*+ column is an exception, being determined not from *im*+/*im*+ tissues, but from green sectors of *im/im* leaves.

† Determined from white sectors of *im/im* leaves.

‡ Substrate phenolphtalein phosphate pyridine salt, buffer Tris pH 7.2, incubation 3 hours at 37°C, activity expressed as an increase in optical density at 400 mμ in a Beckman DU spectrophotometer.

§ Substrate *p*-nitrophenyl thymidine phosphate, buffer Tris pH 7.2, incubation one hour at 37°C, activity expressed as an increase of optical density at 540 mμ in a Beckman DU spectrophotometer.

‖ The crude enzyme extract was heated to 60°C for 10 minutes and incubated in pH 7.5 Tris buffer at 37°C for 2 hours. The data of the chemical analyses are given as percent of dry weight. Enzyme activity is compared on the basis of equal amount of protein.

The calcium and magnesium contents of the normal dark green type and a highly variegated *im/im* mutant were also determined. Since the mutant sample contained approximately one third as much chlorophyll as the wild type, one might expect a comparably lower amount of magnesium in the plants of *im/im* genotype. However, the magnesium and calcium contents are practically identical in the mutant and the normal green wild type (Table 1, Experiments 3, 4). In another study, 40% of the total magnesium and calcium content of bean and tobacco leaves was found in the chloroplasts (STOCKING and ONGUN 1962). Apparently the variegation, which interfered with plastid differentiation (Figure 2) and consequently with leaf pigment synthesis, did not affect the uptake and utilization of these two metals, as it did the metabolism of phosphorus.

According to GRANICK (1963) 35 to 55% of the dry weight of the chloroplasts is protein. Most of this is in an insoluble form. More than half the total nitrogen content of the leaf cells is expected to be in the chloroplasts (STOCKING and ONGUN 1962). Numerous determinations of the soluble protein content exposed small differences between the plants of *im* and *im*+ genotypes (Table 1, Experiment 5).

It appears therefore that in the mutant *im/im*, plastid differentiation, a highly complex process, is affected in a very specific way.

Enzyme studies: Phosphorus may accumulate in the cells if phosphatases, abundant in all tissues, were unrepressed or induced. Neither phosphomonoesterase nor phosphodiesterase activity is different in the two genotypes (Table 1, Experiments 6, 7). Though phosphodiesterases do not produce free inorganic

phosphates, their products may be acted upon by unspecific enzymes which cleave phosphorus bonds.

In another study, the activity of a few phosphatases was assayed from both genotypes. In order to partially inactivate the heat sensitive phosphomonoesterases, the crude enzyme extract was heated and centrifuged again before incubation. The 3′-nucleotidase is a fairly heat stable enzyme without much difference between the two genotypes in its activity. Actually, the enzyme of the mutant seems to be less active (Table 1, Experiment 10). This may be attributed to the inhibitory effect of the increased amount of phosphorus in the mutant tissues. That phosphatases are subject to product inhibition is well known (MORTON 1955).

The chloroplasts contain a significant amount of ribonucleic acid (PARK 1965). WOLLGIEHN and PARTHIER (1964) demonstrated that kinetin prevents RNA breakdown and actually promotes RNA synthesis. When kinetin was incorporated into the nutrient medium of aseptically grown plants, the content of leaf pigments in the mutant was increased. These findings were not surprising. Kinetin was effective in the restoration of chloroplast differentiation only at very toxic concentrations. Apparently its effect was not due to differential inhibition of the growth of the two kinds of tissues, since the total pigment content per plant was approximately doubled at a concentration of 10^{-3} M. A kinetin medium of 5×10^{-3} M killed the plants.

SAHAI SRIVASTAVA and WARE (1965) observed that kinetin treatment of barley leaves reduced the level of DNase and RNase. When kinetin was added to the incubation mixture, the activity of neither of these enzymes was affected.

A ribonuclease assay demonstrated a difference between *im* mutant and the im^+ type (Figure 4). The curves of the ribonuclease activity of the mutant and wild type were not identical or parallel through the pH range of 3 to 8. On the basis of an equal amount of protein at pH 8, the activity of RNase was the same in both genotypes. At pH 7, there was a slightly higher activity in the extract of the mutant. In the acidic range the difference was clear, as the mutant displayed definitely higher activity. Within the range of pH 4 to 6 the mutant always displayed the higher RNase activity. Individual samples from different plants varied considerably. Some samples of the mutant exhibited only 25% excess activity over the wild type; in others five to sevenfold increases were observed. This is understandable since a variegated mutant was compared with the stable wild type. If the variegation has any correlation with ribonuclease, such a variega-

TABLE 2

Effect of kinetin on pigment production of mutant im *(rosettes 29 days old test tube culture)*

Kinetin	No. of plants	Average fresh weight (mg)	Pigment μg per g fresh weight		Pigment μg per plant	
			Chlorophyll	Caroteinoids	Chlorophyll	Carotenoids
Control	20	1.85	50.4 (100%)	19.7 (100%)	0.0504	0.0036
1×10^{-3}	19	0.43	216.3 (429%)	42.8 (317%)	0.0931	0.0182

tion in enzyme activity is also expected. It was observed that the whiter the tissues, the more ribonuclease they contained.

Two questions then arose—whether the increased ribonuclease activity was more or less the specific consequence of the mutant gene function, or whether albinism *per se* was responsible for the increase in amount or activity of the enzyme. To produce enough tissues for biochemical analysis from albina Arabidopsis plants is difficult. DR. E. G. ANDERSON supplied albina maize seedlings, which were almost pigment free, along with some green seedlings. Both types of seedlings were germinated in the sand-benches of the greenhouse. The comparison of the two types of maize did not reveal any difference in ribonuclease activity between the white seedlings and their normal sibs (Table 3). The variation between the data of the two tests was probably due to sampling error. Only a small amount of material (50 mg dry weight) was used for the tests. If there was any variation within the individual plants or in the sampling, such a fluctuation might result.

The lack of parallelism between the two curves (Figure 4) indicates furthermore that there is more than one RNA-digesting enzyme in the crude extract. There is evidence that the mutant produces an excessive amount of only the enzyme with a lower pH optimum.

Free ribonuclease is known to occur in the cytoplasm of higher plants, but several organelles also harbor ribonucleases. By differential centrifugation, two cellular fractions were prepared. The chloroplasts and nuclei were sedimented at 2,500 $\times$ g and processed as indicated in METHODS. The other portion was subjected to 112,000 $\times$ g for 90 minutes, and the supernatant was considered as the nonparticulate fraction.

The chloroplasts plus the nuclei and the nonparticulate fractions were assayed for ribonuclease within the range of pH 4.5 to 8.5. The nonparticulate cellular fraction displayed an optimum around pH 6, while the organelle fractions (nuclei, chloroplasts, mitochondria, ribosomes) contained an enzyme with a definitely higher optimum, similar to that of the chloroplast enzyme (Figure 5). These experiments demonstrate that the mutant is overproducing the enzyme, which is apparently not bound tightly to the organelles.

Since most of our assays were carried out with crude extracts, it was important to know whether the RNA digestion in vitro was carried out by ribonucleases or

TABLE 3

Ribonuclease assay on albina maize seedlings (0.1 M citrate buffer pH 4.6, yeast RNA substrate)

Genotype	Ribonuclease activity	
	Experiment 1	Experiment 2
1351 albina	.094	.121
1351 green sibs	.146	.103
1441 albina	.107	.093
1441 green sibs	.095	.111

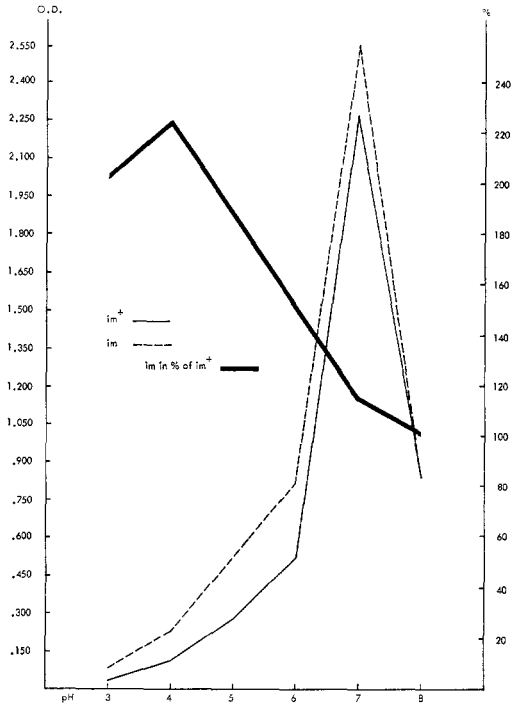

FIGURE 4.—Ribonuclease activity of the wild type and of the mutant in McIlvain's buffer. Crude extracts (390 μg protein per 0.5 ml buffer) were incubated at 37°C with yeast RNA substrate (1.5 mg per 0.5 ml buffer). The reaction was stopped with 3 ml tertiary butanol-acetic acid mixture (2:1, v/v) then chilled for 10 minutes and centrifuged at 10,000 rpm for 10 minutes. The supernatant was diluted fourfold and the optical density of the soluble material was determined in a Beckman DU spectrophotometer at 260 mμ. To assure good measurements throughout the whole pH range, the time of incubation was properly adjusted within the linearity range. The heavy solid line indicates enzyme activity of the *im/im* mutant in percentage of *im*+/*im*+.

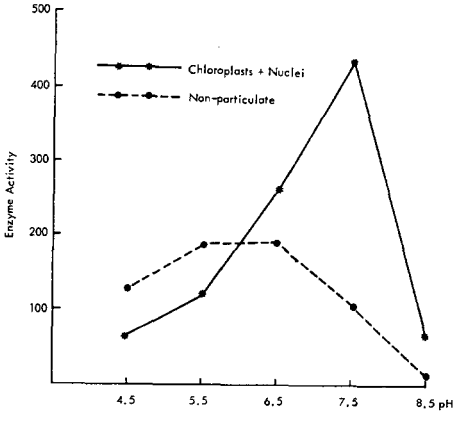

FIGURE 5.—Ribonuclease activity of two cellular fractions in McIlvain's buffer. The different pH optima are evident. The data do not give quantitative estimates on total ribonuclease activity in the two fractions but the curves indicate ribonuclease activity per equal amount of protein.

by phosphodiesterases. Arabidopsis phosphodiesterase is not active at the lower pH values. Since phosphodiesterase is inhibited by PO_4, the RNase assay was performed in citratephosphate buffer. The phosphodiesterase was assayed with *p*-nitrophenyl-thymidine-5′-P which is not digested by RNase. It is known that Mg^{++} ions are necessary for the activity of venom phosphodiesterase (RAZZEL and KHORANA 1959), therefore the diesterase was assayed also in the presence of 1.7×10^{-3} M $MgSO_4$.

On the basis of an equal amount of protein, there was about twice as much phosphodiesterase activity in the soluble cellular fraction as in the fraction with chloroplasts plus nuclei (at pH 7.5). Ribonuclease, on the contrary, was relatively more abundant in the organelle fraction when assayed at pH 7.5. Thus in the nonparticulate fraction the RNA was digested at the higher pH values primarily by the phosphodiesterase. In the particulate fraction, apparently RNase has the major role in RNA disposal (Figure 6).

Attempts to differentiate qualitatively the acid ribonuclease of the mutant from that of the wild type have not been successful.

DISCUSSION

Not much is known about mutants with altered ribonuclease synthesis or activity. The commonly used isolation techniques in microbial systems have failed to

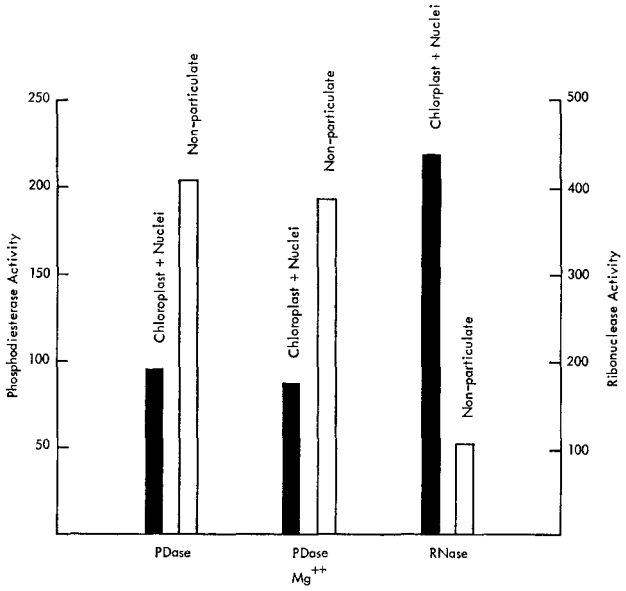

FIGURE 6.—Comparison of phosphodiesterase (pH 7.5 Tris buffer) and ribonuclease (pH 7.5 McIlvain's buffer) activity of the two subcellular fractions. Ribonuclease assay performed as indicated at Figure 4. Phosphodiesterase was assayed with 0.25 ml enzyme, 0.25 ml substrate (1.2 mg *p*-nitrophenyl thymidine-5′-P per 1 ml buffer) and 0.25 ml H_2O or 5×10^{-3} M $MgSo_4$. The mixture was incubated 1 hr, diluted with 2.25 ml H_2O, and optical density was determined at 400 mμ in a Beckman DU spectrophotometer.

identify the mutants even if these types have occurred. In *Escherichia coli*, however, a mutant lacking ribonuclease has been studied (GESTELAND 1966). Since this manuscript has been submitted for publication, two papers reporting increased ribonuclease activity in mutant opaque-2 of maize appeared in print (DALBY and DAVIES, Science 155: 1573; WILSON and ALEXANDER, ibid. 575).

Plant ribonucleases appear especially attractive for genetic studies. Most higher plants contain several ribonucleases (WILSON 1963; GOLASZEWSKI and SZARKOWSKI 1964). In addition, plant ribonucleases generally digest the diester bonds of all four nucleotides and most of them hydrolyze the cyclic phosphates of all four bases. Differences between species of higher plants concerning the catabolic functions of RNase have also been detected (REDDI 1958; SHUSTER and KHORANA 1958; etc.). Variations are obvious among ribonucleases of widely different organisms (cf. JOSEFSSON and LAGERSTEDT 1962). In addition to the types of catabolic functions, the synthetic ability of RNase has also been demonstrated. The sites active in the degradative and synthetic processes are different in pancreatic RNase (BERNFIELD 1965). The wealth of biochemical information available on these enzymes may aid genetic studies if mutants can be produced which are affected in any of these functions. Furthermore, RNases may have an important role in the catabolism of messenger RNA or in controlling other steps of the protein synthesizing machinery (TAL and ELSON 1963; ARTMAN and ENGLEBERG 1964; ANRAKU and MIZUNO 1965).

Ribonuclease activity varies during ontogenesis both in plants (LEDOUX, GALAND and HUART 1962; HANSON, WILSON, CHRISPEELS, KRUEGER and SWANSON 1965) and in animals (BRESNICK, SAGE, and LANCLOS 1965). Thus differentiation, organization and growth may be partly controlled by ribonucleases. The genetic control of development may be exercised through variants of the protein, through differential activation, through the suppression of one or several enzymes with similar or different function, or through the subcellular localization of the enzymes. Genetic amino acid variants of mammalian ribonucleases have been identified (ANFINSEN 1959). Special natural inhibitors of RNase have been revealed both in plants (BERNHEIMER and STEELE 1955) and in animals (ROTH 1957; SHORTMAN 1961). RNases with different pH optima in different tissues (WILSON 1963) or different organelles of plants have also been reported (GOLASZEWSKI and SZARKOWSKI 1964).

Thus ribonucleases may play some role in the genetic control of differentiation. In Arabidopsis, variegation in plastid formation is associated with the activity of one of the ribonucleases.

The mutant *im* has a facultative ability to produce normal chloroplasts (Figure 2); therefore, the mutation must not have affected a "structural gene". Furthermore, attempts to find a qualitative difference between the enzyme of the mutant and wild type were unsuccessful. Some kind of a controlling factor has seemingly been mutated. Since the *im* gene is recessive, this controlling factor —by definition—cannot be the regulator gene of an operon.

RNase synthesis, rather than RNase activation, seems more likely to be involved because the activity of the wild-type and mutant enzymes could not be affected

differentially in vitro. In the green tissues of the mutant, however, apparent normal activity was detected. The effect of chemical treatments (e.g. azapyrimidines), which modified the expression of the mutant, could be observed after a lag period. Modification of the variegation is easiest by physical agents during germination and in the early stages of development when the synthesis of RNase is rapid.

The activity of the mutant gene is strictly cell-limited. Single green cells on a white background or single white cells in a green tissue are frequently observed. This indicates that a macromolecule is involved which does not diffuse through cell membranes. Whether some primary gene product or a corepressor or an inducer is directly responsible for the variegation is not known. It would be interesting indeed to know whether the chloroplast ribonuclease is controlled by the plastom.

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SUMMARY

The recessive mutant *im* in homozygous condition displays variegation. In certain cells plastid differentiation is arrested; in others the chloroplasts are apparently normal. Within a single cell all the plastids develop uniformly, and the distribution of the two types of cells in the tissues is not random. The prevailing epigenetic factors determine the transition from one state of differentiation to the other. The sexual progeny of the two types of sectors is indistinguishable, and both repeat the variegation. Heterozygotes never reveal any sectoring; an interpretation of high somatic mutability can not be invoked. Since the mutant cells have a facultative ability to produce normal chloroplasts, the genetic alteration did not affect directly any of the "structural genes." Mutation in a "regulator gene" is ruled out because *im* is recessive. Direct evidence for the existence of an "operator site" is not available.—The white tissues of the mutant accumulate inorganic phosphorus. The calcium and magnesium contents of the two types of plants are the same. Phosphatases are apparently not different in the mutant and wild type. When the mutant is grown on media containing 6-azauridine or 6-azacytidine the variegation is suppressed, and the normal green phenotype is more or less restored. The effect of kinetin, a regulator of RNA metabolism is similar. In the variegated mutant there is an excessive ribonuclease activity. The accumulated phosphorus is apparently a by-product of the increased RNA catabolism. Arabidopsis possesses three ribonucleases. Only an acid ribonuclease is overproduced in the mutant. This enzyme is not associated with the chloroplasts. Apparently the *im* locus has an important role in the control of the synthesis of this ribonuclease.

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