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Movement of ^{14}C-compounds from Maternal Tissue into Maize Seeds Grown *in Vitro*[1,2]

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ABSTRACT

Uptake from nutrient media into the cob and translocation of various ^{14}C-compounds from maternal tissue (cob) into developing maize seeds was examined by using caryopsis cultures. Based on relative ^{14}C concentrations in the cob and the endosperm, it was concluded that the relative efficiencies of movement of amino acids (leucine, phenylalanine, proline), vitamins (thiamine HCl, nicotinic acid), and nucleic acid bases (adenine, thymine) from the cob to the endosperm were 11 to 250 times lower than that of sucrose. Thiamine was unique in that it was concentrated in the embryo at a level that was almost 10 times higher than in the endosperm. The absence of auxotrophic mutants requiring an organic supplement in higher plants (other than thiamine auxotrophs) may be explained by inadequate translocation of these essential metabolites into the mutant zygotes (embryos) to enable their development to mature seeds.

Auxotrophic mutants in higher plants are rarely isolated following mutagenic treatment of either seeds or pollen, and those that have been isolated and confirmed as true auxotrophs requiring an organic supplement for growth are all thiamine-requiring (13). A nitrate reductaseless mutant of *Arabidopsis thaliana* requiring ammonium as a nitrogen source has also been isolated following seed treatment with nitrosoguanidine (2, 12). Since Langridge (7) discovered a temperature-sensitive thiamine auxotroph in *Arabidopsis*, approximately 200 independently derived thiamine auxotrophs have been detected at four loci in this species. (13) However, other auxotrophs are conspicuously missing. For instance, although Redei and his colleagues (9) found 42 thiamine mutants by examining 128,226 haploid genomes which had been treated with either ethylmethane sulfonate or x-rays, no other auxotrophs were detected in these experiments. Thiamine mutants have been also discovered in tomato (8). A pantothenate-requiring cell line of *Datura innoxia* has been induced in cell culture (14), as have nitrate reductaseless cell lines of tobacco (10). The isolation of the pantothenate-requiring *Datura* cell line indicates that auxotrophic plant mutants requiring organic supplements other than thiamine can be isolated in cell cultures.

The tacit assumption of an investigator applying a mutagenic treatment to either seeds or pollen is that, in an advanced generation, seeds homozygous for an induced, auxotrophic mutant will be supplied by the heterozygous plant on which they are borne with the essential metabolite in adequate quantity so that the mutant zygotes can develop to mature dormant seeds. Subsequent attempts to grow plants from such mutant seeds then would reveal that the plants were incapable of autotrophic growth, and the investigator could proceed to ascertain that a particular supplementation to a basic growth medium would sustain normal or nearly normal growth. Several hypotheses (4, 11) have been proposed to account for this apparent lack of auxotrophs in higher plants following such mutagenic treatments. One of these hypotheses is that there is a selective elimination of mutant zygotes due to inadequate translocation of essential compounds from maternal tissue to developing seeds.

We report the results of a test using caryopsis culture of maize to ascertain whether thiamine is translocated more readily to the developing seed or is apportioned differently within the seed than some other compounds for which auxotrophic mutants might be expected but have not been found.

MATERIALS AND METHODS

Plant Material. Maize (*Zea mays* L.), inbred W22 grown in the field and self-pollinated, was used here. The technique and medium for caryopsis culture were those of Gengenbach (5, 6).

Ears of maize (5 days after pollination) were surface-sterilized in 20% (v/v) Clorox and cut into cob blocks containing four caryopses. Five of these blocks were placed on the medium (50 ml) in a 125-ml Erlenmeyer flask in such a way that only cob tissue was in contact with the medium. They were grown for 10 days on the standard medium and then transferred onto ^{14}C-containing medium. After a 7-day incubation at 28 ± 1 C in the dark, the five cob blocks within a flask were bulked, frozen on dry ice, and stored at −20 C until analysis.

The sucrose concentration of Gengenbach's medium was modified to 5% (w/v), which supported good growth of caryopses for up to 2 weeks. All the experiments were carried out with duplicate flasks, except for the fructose experiment in which one of the flasks was lost due to microbiological contamination.

Radioactive Chemicals. [U-^{14}C]Sucrose (673.0 mCi/mmol), D-[U-^{14}C]fructose (188.0 mCi/mmol), L-[U-^{14}C]leucine (54.7 mCi/mmol), L-[U-^{14}C]phenylalanine (485.0 mCi/mmol), L-[U-^{14}C]proline (282.5 mCi/mmol), [8-^{14}C]adenine (56.2 mCi/mmol), and [2-^{14}C]thymine (53.2 mCi/mmol) were purchased from New England Nuclear. [Thiazole-2-^{14}C]thiamine HCl (24.3 mCi/mmol) and [carboxyl-^{14}C]nicotinic acid (61.0 mCi/mmol) were purchased from Amersham. All radiochemicals were dissolved in distilled H_2O, filter-sterilized, and added to autoclaved medium. The amounts of radioactive compounds in the media are shown in Table I.

Radioactive Assay. The frozen blocks were thawed and divided into four tissue groups: embryo, endosperm, pericarp and pedicel, and cob. They then were dried at 70 C, weighed, and ground to

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powder by mortar and pestle. Two to 5 mg of each tissue were mixed with 10 ml of a mixture of toluene-PPO-dimethyl POPOP scintillator and scintillation grade Triton X-100 (2:1), shaken well, left for 24 h at 4 C, and counted in a liquid scintillation counter (16). Counts were corrected for different counting efficiencies due to tissue and sample weight differences. All counts were made with duplicate vials.

Paper Chromatography. Dried tissue powder (20–50 mg) was extracted with 0.8 ml of either water or 5% (w/v) trichloroacetic acid. The extracts were occasionally concentrated by vacuum evaporation at 50 C. Whatman 3MM paper was used for all separations. Solvents used were 1-butanol-ethanol-H_2O (13:8:4), 1-butanol-acetic acid-H_2O (12:3:5), and 1-propanol-H_2O (4:1) for separations of sugars, amino acids, and nucleic acid bases, thiamine HCl, or nicotinic acid, respectively. Spots were cut out and counted in 10 ml toluene-PPO-dimethyl POPOP scintillation fluid in a liquid scintillation counter.

RESULTS

Total Uptake of ^{14}C-compounds by Cultured Cob Blocks. Total uptake of ^{14}C-compounds by cultured cob blocks is summarized in Table I. The specific radioactivities of ^{14}C-compounds in the medium were all similar except those for [^{14}C]sucrose and thiamine. The low specific radioactivity of [^{14}C]sucrose was due to the large amount of unlabeled sucrose required for the normal growth of maize caryopses *in vitro*. Thiamine is also a constituent of the nutrient medium.

The tested compounds can be grouped into three distinct classes according to relative efficiencies of uptake into the cultured cob block, as summarized in the last two columns of Table I. The first class of compound which was most efficiently taken up into the cultured tissue includes the three amino acids, adenine, and nicotinic acid. The second group with intermediate uptake consists of fructose and thiamine HCl. Thymine, characterized by its extremely low uptake, constitutes the third class. The relative efficiency of uptake for this compound was 50 times less than those compounds in the first class. Because the amount of sucrose in the medium was approximately 5 orders of magnitude higher than other compounds, this compound was not placed into the three classes mentioned above.

Distribution of ^{14}C among Tissues in Cultured Cob Blocks. Table II shows the distribution of ^{14}C in various tissues of the cultured cob blocks both as total ^{14}C content and ^{14}C content/mg dry weight. As is shown, each compound had a characteristic pattern of ^{14}C distribution between different tissues. For instance, sucrose and fructose had a gradient of increasing ^{14}C concentration in the order of cob-pericarp and pedicel-endosperm-embryo when stated as cpm/mg dry weight. However, for leucine and phenylalanine, this order was reversed.

To convey the relative concentrations of ^{14}C in various tissues, ratios of ^{14}C concentrations are summarized in Table III. For example, the endosperms had approximately 5 times more counts than did the cob per mg dry weight when [^{14}C]sucrose or [^{14}C]-fructose was supplied. Conversely, the cob accumulated 10 to 50 times greater amounts of ^{14}C than the endosperms did when amino acids, adenine, or vitamins were supplied.

When the ratios of ^{14}C concentrations between the endosperm and the embryo were examined, the unique aspect of thiamine became apparent. The quantity of this compound was almost 10 times higher in the embryo than in the endosperm on the basis of cpm/mg dry weight, but the accumulation of thiamine in the embryo is observed also when the data are presented on the basis of biological units.

Conversions of ^{14}C-compounds Taken up by Cultured Cob Blocks. Distribution of ^{14}C among various soluble compounds in extracts from cultured tissues was analyzed by paper chromatography. The results are shown in Tables IV, V, and VI. After the entry into the cob tissue, vitamins appeared to undergo little conversion (Table IV). The counts supplied as nicotinic acid remained in nicotinic acid in the cob tissue. Similarly, approximately 80% of total ^{14}C in cob extracts was recovered as thiamine. This was also true in the endosperm and embryo extracts. There was substantial conversion of adenine and thymine in the cob and endosperms to the corresponding phosphorylated nucleosides.

In contrast to vitamins and nucleic acid precursors, amino acids were often converted to other compounds within the cob tissue (Table V). Approximately 20% of total ^{14}C activity in the cob and endosperm extracts was recovered as the amino acid supplied. In addition, the ^{14}C label was also found in other amino acids and in sugars. The pattern of ^{14}C distribution among various compounds in the endosperm extracts was similar to that in the cob extracts.

As expected, sucrose and fructose were converted to a variety of compounds in cob tissue, although 16 and 27% of ^{14}C was still found in simple sugars when [^{14}C]sucrose and [^{14}C]fructose, respectively, were supplied (Table VI). Also, significant proportions of the label were found in amino acids.

DISCUSSION

The results suggest that the movement of the vitamins, thiamine-HCl, and nicotinic acid from the cob to the endosperm is strongly restricted in *in vitro* conditions. Nucleic acid bases and their phosphorylated nucleosides appeared similarly prevented from entering into the endosperm through the cob tissue.

Because of extensive metabolic conversions in the cob during

Table I. *Total ^{14}C Uptake by Cultured Cob Blocks*

Labeled Compound	Total ^{14}C Added	Total Amounts	Total ^{14}C Taken Up		Total Uptake		Total Uptake	
			Flask 1	Flask 2	Flask 1	Flask 2	Flask 1	Flask 2
	cpm × 10^{-6}/flask	*nmol/flask*	*cpm × 10^{-4} flask*		*nmol/flask*		*nmol/g dry wt*	
Sucrose	27.8	7.3×10^6	18.0	17.3	4.7×10^4	4.5×10^4	1.1×10^5	1.0×10^5
Fructose	23.1	62	51.2		1.38		2.7	
Leucine	16.5	152	163.9	161.7	16.39	16.17	41.0	29.4
Phenylalanine	28.7	30	383.4	454.4	4.00	4.73	7.8	9.0
Proline	10.8	19	103.9	126.6	1.82	2.22	4.2	5.7
Adenine	11.9	107	190.2	185.2	17.29	16.84	31.9	31.0
Thymine	13.5	128	3.9	3.9	0.35	0.35	0.7	0.8
Thiamine	11.2	289	23.3	41.6	7.49	5.97	13.9	20.7
Nicotinic acid	9.1	75	105.6	110.5	8.80	9.21	20.1	18.8

Table II. *Distribution of ^{14}C Label in Various Tissues after in Vitro Seed Development*

The media contained different labeled compounds.

Labeled Compound	Flask	Total ^{14}C Content				^{14}C Content			
		Cob	Pericarps + pedicels	Endosperms	Embryos	Cob	Pericarps + pedicels	Endosperms	Embryos
		cpm × 10^{-4}				*cpm mg^{-1} dry wt*			
Sucrose	1	2.14	4.87	9.65	1.28	174	276	818	2,024
	2	2.47	4.29	9.15	1.26	169	246	845	2,854
Fructose	1	5.19	8.92	33.09	4.04	378	497	1,804	3,423
Leucine	1	112.11	39.47	11.70	0.61	11,927	2,602	1,083	660
	2	110.11	35.34	15.46	0.75	9,875	1,860	828	631
Phenylalanine	1	285.86	68.97	27.48	0.98	19,526	3,964	1,476	1,061
	2	316.00	87.79	47.67	2.95	19,812	5,487	2,501	1,890
Proline	1	70.38	26.58	6.43	0.50	6,860	1,860	363	437
	2	87.65	34.50	3.89	0.57	7,504	2,355	342	405
Adenine	1	125.27	60.92	3.74	0.34	8,209	3,626	183	210
	2	131.55	50.68	2.70	0.33	7,504	2,762	161	203
Thymine	1	1.61	1.39	0.74	0.16	104	83	53	121
	2	1.97	1.11	0.61	0.19	124	70	49	144
Thiamine	1	12.86	8.19	1.24	1.02	1,310	553	74	683
	2	24.10	13.99	1.94	1.64	1,634	952	95	953
Nicotinic acid	1	46.47	51.51	6.40	1.20	3,642	2,969	518	876
	2	54.10	46.15	9.13	1.23	3,990	2,833	506	1,061

Table III. *Ratios of ^{14}C Content in Various Tissues*

Labeled Compound	Flask	Ratio of ^{14}C Contents in			$\bar{X}$[a]
		Endosperm/cob	Embryo/endosperm	Embryo/endosperm	
		cpm mg^{-1} dry wt		*biological units*	*nmol mg^{-1} dry wt embryo × 10^3*
Sucrose	1	4.70	2.47	0.15	
	2	5.00	3.38	0.18	642,000
Fructose	1	4.77	1.90	0.12	9.2
Leucine	1	0.09	0.72	0.05	
	2	0.08	0.76	0.05	5.9
Phenylalanine	1	0.08	0.74	0.04	
	2	0.13	0.74	0.06	1.5
Proline	1	0.05	1.20	0.09	
	2	0.05	1.18	0.10	0.7
Adenine	1	0.02	1.15	0.08	
	2	0.02	1.26	0.10	1.9
Thymine	1	0.51	2.28	0.20	
	2	0.40	2.94	0.35	1.2
Thiamine	1	0.06	9.23	0.73	
	2	0.06	10.04	0.79	21.0
Nicotinic acid	1	0.14	1.69	0.19	
	2	0.14	2.10	0.15	8.0

[a] $\bar{X}$ = mean value.

Table IV. *Distribution of ^{14}C Supplied by Nitrogen Base or Vitamin among Various Compounds in Tissue Extracts*

Labeled Compound	Tissue	Total ^{14}C in Extracts		
		Original form	Phosphorylated nucleosides	Total
			%	
Adenine	Cob	31.0	55.2	86.2
	Endosperm	43.9	56.1	100.0
Thymine	Cob	52.6	15.5	68.1
	Endosperm	34.3	27.3	61.6
Thiamine HCl	Cob	70.9		
	Endosperm	85.1		
	Embryo	81.0		
Nicotinic acid	Cob	100.0		

the culture period, the movement of amino acids should be evaluated with caution. The compounds derived from an amino acid taken up into the cob could be grouped into two classes. The first class includes those compounds which have similar or lower relative efficiencies of translocation from the cob to the endosperm. Presumably, some amino acids derived from the originally labeled one would be in this class. These accounted for 15.3, 15.4, and 29.5% of the label for leucine, phenylalanine, and proline, respectively. Also, intermediates in catabolic processes and products of deaminations could possibly be included in this class. This class of compounds would be a major class of derived labeled compounds and would not considerably affect the apparent efficiency of translocation of the amino acid originally supplied.

The second class of compounds derived from a labeled amino acid would be those that are more easily transported into the endosperm through the cob. Sucrose would be included in this category and 9.2, 2.1, and 28.3% of total ^{14}C in the cob extracts was found in sucrose plus glucose and fructose for leucine, phenylalanine, and proline, respectively. These derivatives increase the apparent efficiency of movement of a labeled amino acid. The relative efficiencies of movement from the cob to the endosperm calculated from the relative proportions of ^{14}C labels in these tissues could possibly be overestimates owing to conversions in the cob. Therefore, we concluded that there is also a strong barrier to movement between the cob and the endosperm for these amino acids in *in vitro* conditions.

Sucrose, the major form in which photosynthate is translocated in higher plants, is also converted into other compounds, some of which are not loaded into the phloem. Fructose, on the other hand, is phloem-immobile (18), and fructose probably is translocated only after its incorporation into sucrose. The data in Table VI showing that, when labeled fructose is supplied in the medium, nearly as much label is found in glucose as in fructose in the endosperms are consistent with the view.

In considering the possible significance of the results presented

Table V. *Distribution of ^{14}C Originating in Amino Acids among Various Compounds in Tissue Extracts*

^{14}C Compound Added to Medium	Tissue	Total ^{14}C in Extracts							
		Original form	Other amino acids	Subtotal	Sucrose	Glucose	Fructose	Subtotal	Total
					%				
Leucine	Cob	29.5	15.3	44.8	3.1	2.2	3.9	9.2	54.0
	Endosperm	31.4	9.9	41.3	4.9	3.8	6.1	14.8	56.1
Phenylalanine	Cob	23.9	15.5	39.3	3.9	3.3	13.9	21.1	60.4
	Endosperm	23.2	13.6	36.3	5.7	4.0	5.3	15.0	51.8
Proline	Cob	18.7	29.5	48.2	10.9	10.9	6.5	28.3	76.5
	Endosperm	31.4	34.5	65.9	6.6	7.6	5.9	20.1	86.0

Table VI. *Distribution of ^{14}C Initially Present in Sugars among Various Compounds in Tissue Extracts*

Labeled Compound	Tissue	Total ^{14}C in Extracts					
		Sucrose	Glucose	Fructose	Subtotal	Amino acids	Total
				%			
Sucrose	Cob	4.7	2.3	9.4	16.4	24.1	40.5
	Endosperm	10.0	12.7	15.8	38.5	42.3	80.8
Fructose	Cob	6.0	2.9	17.9	26.8	27.7	54.5
	Endosperm	8.0	11.3	13.2	32.5	36.0	68.5

here to the striking rarity of auxotrophic mutants in higher plants, it should be remembered that the *in vitro* conditions employed are quite different from those *in vivo*. Arruda and Da Silva (1) found all the amino acids present in proteins except proline and cystine in the vascular sap extracted from the shank subtending developing maize ears, with glutamine being the major component (29 mol % at 14 days after pollination). Sodek and Wilson (15), who injected [^{14}C]leucine into the shank of developing maize ears, found that, although times of injection and harvest were varied, generally about 15% of the leucine label was recovered in the endosperms. The embryos contained a "considerable but undetermined amount of label" (15). In both flasks 1 and 2, where labeled leucine was present, 4.9% of the radioactivity present in the seeds was found in the embryos.

It is also possible that some compounds taken up by the cob tissue do not enter pools which are being transported into the developing seed.

Different partitioning of the various molecules tested between embryo and endosperm cannot be explained by partitioning into different translocation pathways to the embryo and endosperm from maternal tissue. In maize, there are no vascular connections between the embryo and maternal tissue. All translocated substances enter the developing seed through specialized transfer cells at the base of the endosperm and then move into the embryo (3, 17).

Assuming that similar restrictions on translocation operate *in vivo*, the results suggest the following points. (*a*) All the compounds tested here, other than sucrose, are subjected to a strong inhibition of transport from maternal tissue to the seeds. This inhibition may be strong enough to prevent the development to mature, dormant seeds of mutant zygotes lacking the capacity to synthesize an essential metabolite, even if adequate quantities were being supplied to the developing ear. (*b*) That thiamine auxotrophs are the only auxotrophs isolated in higher plants by methods that depend on a mutant zygote successfully developing on a heterozygous plant may be explained by the movement of thiamine to the embryo. Although its movement from the cob to the endosperm is no better than that of other compounds tested, this vitamin is accumulated in the embryo preferentially. In addition, the small requirement for vitamins for normal growth, compared with amino acids or nucleic acid precursors, is an additional factor that could explain the existence of thiamine auxotrophs in higher plants.

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