and the abnormal genes would be active in each cell. Some type of intermediate threshold might be anticipated, and this is what was found. Although inactivation of the locus controlling glucose-6-phosphate dehydrogenase (G-6-PD) formation undoubtedly occurs (*6*), it is not at all certain that the entire human X chromosome undergoes a functional inactivation. It has been suggested that in the mouse it does not (*7*).

On the other hand, it is also possible that inactivation of the protanopic locus occurs, but that the patches of inactivation are so small that a 1-degree target covers such a large area that it gives rise to intermediate threshold values. It was originally suggested (*8*), on the basis of the very early appearance of the chromatin body (*9*), that the number of cells present at the time of inactivation was quite small. However, recent evidence based on electrophoretic variants of G-6-PD indicates that the stage of development at which inactivation takes place may be a much later one (*10*).

Thus, the present studies exclude inactivation of the protan locus of the X chromosome if the inactivated patches are relatively large. However, only techniques which could detect the activity of this gene in small areas containing only a few cone receptors (perhaps even one) would be sufficient to exclude inactivation of the protan locus if the patches are minute.

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Ribosomal-RNA Synthesis in the Absence of Ribosome Synthesis in Germinating Cotton Seeds

Abstract. *Cotton embryos after 72 hours of germination synthesize ribosomal RNA that does not become incorporated into ribosomes and that is very stable. This RNA has many characteristics similar to those of newly synthesized ribosomal RNA of bacteria.*

During the initial phase of their germination cotton seeds synthesize ribosomes as demonstrated by their ability to incorporate ^{32}P-labeled phosphate into ribosomal RNA (rRNA) and ribosomes (*1*). The rRNA synthesized at this period does not contribute significantly to the rRNA present in the mature seed and protein synthesis during this initial phase of germination is apparently catalyzed by ribosomes and stable messenger RNA present in the mature seed (*1*).

We now report that during cotton-seed germination the synthesis of ribosomes ceases at later stages of germination, but that a low level synthesis of rRNA persists. This rRNA is extremely stable but does not become incorporated into ribosomes possibly because of the absence of ribosomal protein synthesis at this point in germination.

In order to follow RNA synthesis during germination, cotton seeds were germinated for varying lengths of time at 30°C in sterile soil and harvested; and the cotyledons were separated from the hypocotyl. The cotyledons were then infiltrated in a vacuum desiccator for 30 minutes with a solution of carrier-free $K_2H^{32}PO_4$ (100 μc/ml); they were then washed thoroughly and placed on moist filter paper for further incubation. All of these operations were carried out in the dark except as otherwise stated.

The preparation of ribosomes and polyribosomes, and of ribosomal RNA, has been described, as have the methods for obtaining the sucrose density-gradient profiles of ultraviolet absorbancy and radioactivity (*1*). In addition, rRNA from the pellet resulting from centrifuging the crude homogenate at 25,000*g* for 20 minutes was purified and characterized with regard to ultraviolet absorbancy and radioactivity on a sucrose density gradient (5 to 20 percent). This pellet contains mitochondria, proplastids, and cellular debris in addition to nuclei, but the extracted RNA is referred to as nuclear RNA. In order to extract rRNA instead of DNA and DNA-like RNA, this pellet was resuspended in 0.1*M* acetate buffer, *p*H 6.0, 0.1*M* NaCl, and bentonite (1 mg/ml); to this suspension was added 1 volume of redistilled phenol saturated with the above buffer solution. After being shaken in the cold the phases were separated by centrifugation, and the aqueous layer was diluted with two volumes of ethanol; the precipitated RNA was collected and suspended in 0.01*M* tris-succinate buffer, *p*H 7.5, 0.001*M* EDTA (ethylenediaminetetraacetate), and bentonite, and reprecipitated by modifying the solution to 1 percent cetyltrimethylammonium bromide (*2*). This RNA was reconverted to the soluble sodium salt and sedimented on a 5 to 20 percent sucrose density gradient containing 0.01*M* tris-succinate, *p*H 7.5, 0.001*M* NaCl, and 0.001*M* EDTA.

Cotton embryos during the first 16 hours of germination incorporate a considerable amount of isotope into ribosomes and polyribosomes (Fig. 1*a*). When these ribosomes and polyribosomes are treated with sodium dodecyl sulfate (SDS) to dissociate rRNA from ribosomal protein components (*3*) and fractionated on a sucrose density gradient, the radioactivity peaks coincide with the absorbancy peaks (Fig. 1*b*); this indicates that the radioactivity incorporated into ribosomes is incorporated into both heavy and light rRNA. When embryos which have germinated in the dark for 72 hours are infiltrated with isotope and then incubated for 3 hours more, the density-gradient profile of isotope incorporation into the ribosomes is markedly different. Although the absorbancy profile is similar to that obtained from ribosomes from 16-hour germinated embryos, virtually no incorporation of isotope into ribosomes has taken place during the 3-hour incubation after isotope infiltration (Fig. 2*a*). Rather, the only substantial incorporation is into material sedimenting more slowly than the 80*S* monomeric ribosome and is not associated with any pronounced absorbancy peak.

Table 1. Nucleotide composition (molar percentage) of RNA. AMP, adenylic acid; GMP, guanylic acid; CMP, cytidylic acid; UMP, uridylic acid; TMP, thymidylic acid.

AMP	GMP	CMP	UMP(TMP)
	Light rRNA		
22.9	30.1	21.8	25.1
	Light precursor RNA from ribosomes		
22.9	32.3	21.5	23.3
	Light precursor RNA from nucleus		
23.0	31.6	21.9	23.6
	Heavy rRNA		
21.2	32.4	23.4	23.0
	Heavy precursor RNA from ribosomes		
22.4	31.7	21.8	23.6
	Heavy precursor RNA from nucleus		
25.4	31.4	19.8	23.4
	sRNA		
17.2	31.0	27.7	24.3
	(DNA)		
33.0	17.0	17.0	33.0

When a portion of the ribosome preparation that is not sedimented on the 10- to 34-percent sucrose gradient is treated with SDS and sedimented on a 5- to 20-percent sucrose gradient, the absorbancy profile characteristic of heavy and light rRNA is obtained. The radioactivity which was not associated with ribosomes or polyribosomes was dissociated by SDS into two peaks which closely, but not precisely, approximate the sedimentation positions of both heavy and light rRNA (Fig. 2*b*).

To determine the nature of this material, the fractions from the gradient of ribosomes that contained the radioactivity were pooled, precipitated with trichloroacetic acid after addition of carrier RNA from yeast, redissolved in buffer, and treated with ribonuclease. The radioactive material was insoluble in acid initially, and was made soluble in acid by treatment with ribonuclease. The base ratios as judged by the radioactivity of fractionated nucleotides were determined for the rRNA fractions, pooled from the sucrose gradient, which contained the peaks of radioactivity (*4*). The labeled RNA species have essentially the same base composition as the two species of cotton rRNA derived from ribosomes (Table 1).

When nuclear RNA was prepared from embryos germinated 72 hours and sedimented through a sucrose gradient, the amount of radioactivity incorporated into RNA was far greater than that found in the ribosomal RNA preparation. The peaks of radioactivity closely match the absorbancy peaks, but here again there was a deviation between the absorbancy peaks corresponding to heavy and light rRNA and radioactivity (Fig. 2*c*). The radioactivity of the light RNA was often but not always greater than that in the heavy RNA in preparations of nuclear RNA. The base composition of the labeled nuclear RNA obtained from the sucrose-gradient peaks was similar to that of the two species of rRNA obtained from ribosomes of cotton (Table 1).

A possible interpretation of the foregoing data would be that, at this stage of germination, the incorporation of ribosomal precursor RNA into ribosomes has essentially ceased or at least is retarded to the extent that a precursor particle tends to accumulate, and that this particle can be dissociated into a heavy and light rRNA having the same base composition as rRNA from cotton but differing slightly in sedimentation properties.

In an attempt to demonstrate the incorporation of this precursor particle into ribosomes, cotyledons from 72-hour germinated embryos were incubated for 6 and 9 hours after isotope infiltration and fractionated. Even after these extended incubations the particle was not incorporated into ribosomes nor was it degraded; rather it continued to accumulate (Fig. 2, *d* and *g*). This accumulation is reflected in the accumulation of radioactivity in the sucrose-gradient profiles of both rRNA (Fig. 2, *e* and *h*) and nuclear RNA (Fig. 2, *f* and *i*).

Since these embryos after 72 hours of germination are placed so as to receive sunlight, exposure to light may trigger the synthesis of those components necessary for the incorporation of this particle into ribosomes. Consequently, seeds were allowed to germinate for 72 hours; the germinated seeds were infiltrated with isotope and incubated for 3 hours in the light. When ribosomes, rRNA, and nuclear RNA were prepared from these embryos and fractionated on sucrose gradients, there was no incorporation of isotope into ribosomes; furthermore, the synthesis of the ribosome precursor particle was totally suppressed by exposure to light (Fig. 2, *j*, *k*, and *l*).

Since exposure to light stops the synthesis of the ribosomal precursor, 72-hour embryos were infiltrated with isotope, incubated for 3 hours in the dark to allow the accumulation of the particle, and further incubated for 3 hours in the light to reveal the fate of the particle under this condition. There was no degradation of the particle nor was the particle incorporated into ribosomes. The amount of isotope associated with the particle was the same after 3 additional hours in the light as it was after the 3 initial hours in the dark.

These observations may be interpreted to indicate that at this stage of germination there is a small amount of synthesis of a remarkably stable particle comprised of "nascent" rRNA that is not incorporated into ribosomes and whose synthesis can be stopped by exposure to light. Despite the fact that the base composition of this RNA is

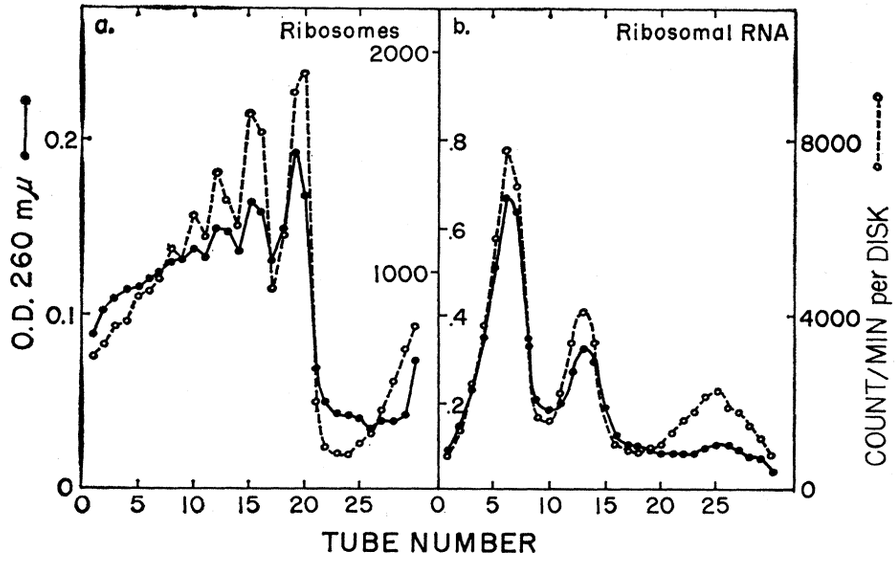

Fig. 1. Sucrose density-gradient profiles of absorbancy and radioactivity from embryos incubated with ^{32}P for 16 hours. (*a*) Ribosomes from embryos germinated 16 hours. (*b*) The SDS-released RNA from ribosomes of embryos germinated 16 hours.

essentially that of RNA derived from cotton ribosomes, the sedimentation characteristic of the heavy RNA from the particle is always distinct from that of the heavy rRNA derived from ribosomes, and that of the light RNA from the particle is usually distinct from light rRNA derived from ribosomes.

In order to rule out the possibility in these experiments that the isotope is being incorporated into the RNA of bacteria infecting these embryos, penicillin and streptomycin were infiltrated into embryos along with isotope. The extent of isotope incorporation into the particle and into its "nascent" rRNA was the same in both the treated and untreated embryos. Likewise the legitimacy of detaching and using only the cotyledons (the storage and initial photosynthetic tissue of cotton embryos) rather than the entire plantlet in these studies was tested by carrying out the same experiments on whole embryos (72 hours germinated) and also on the dissected hypocotyl and root system. In all cases the same build-up of this particle and the unique sedimentation properties of its RNA species were observed.

Scherrer *et al.* (*5*) have reported that, in HeLa cells, both heavy and light rRNA, presumably derived from ribosomes, are initially derived from a precursor with a sedimentation rate approximating 45*S*. We cannot obtain a sedimentation rate for this particle by conventional means since its accumulation is insufficient to contribute an absorbancy peak on the sucrose gradient. From the sucrose gradients, the sedimentation rates were calculated by the method of Martin and Ames (*6*), and the values are close to that of the HeLa cell precursor.

In *Escherichia coli* there is an accumulation of particles whose sedimentation rates are approximately 25 to 30*S*, and these contain both the heavy and light species of *E. coli* rRNA. These particles accumulate under conditions of protein-synthesis inhibition (*7*) or under nutritional conditions that promote a preferential synthesis of rRNA (*8*). These particles are incorporated into ribosomes upon the removal of the inhibitors or supplementation of the growth medium.

In several instances the RNA species isolated from these particles have the

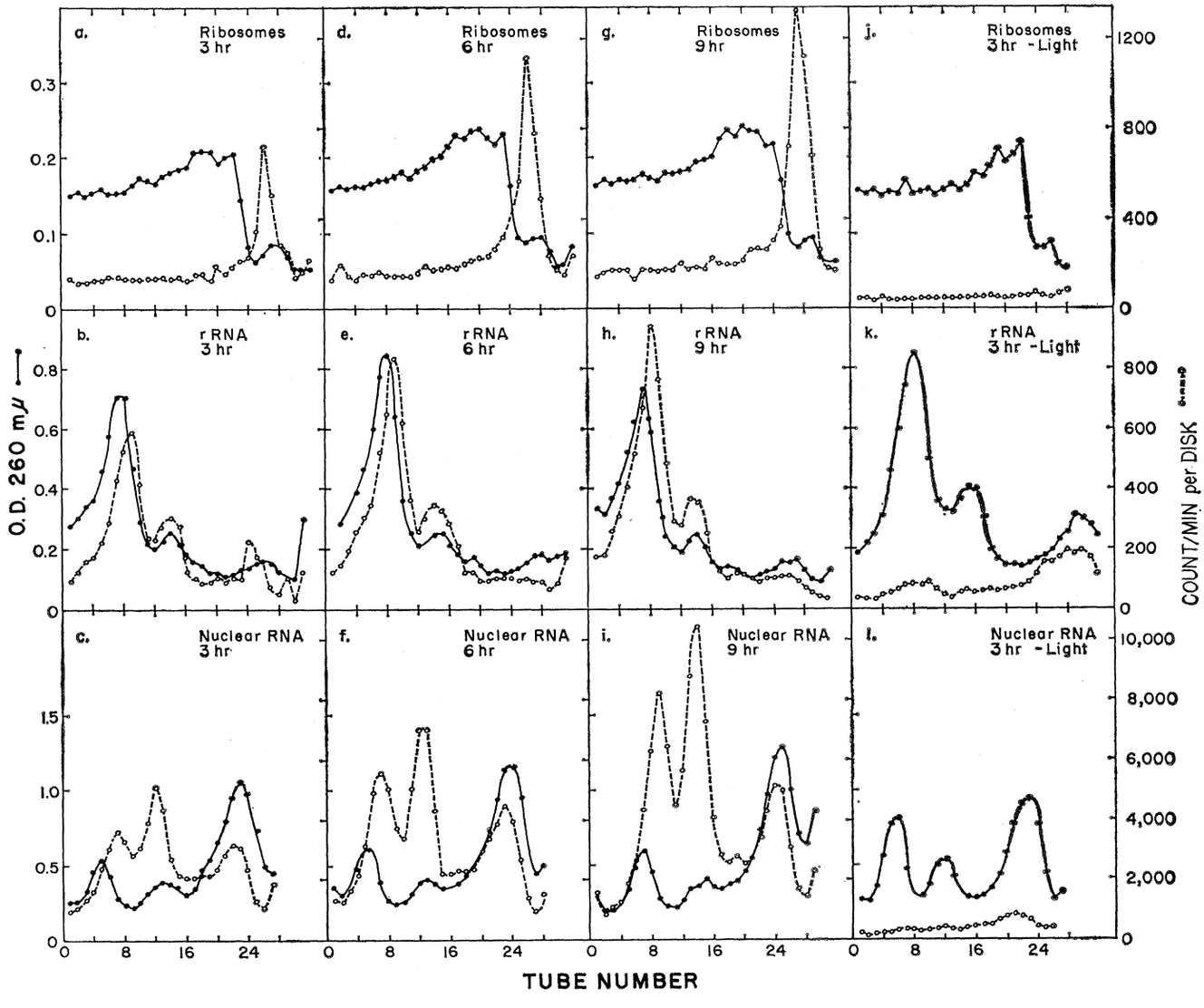

Fig. 2. Sucrose density-gradient profiles of absorbancy and radioactivity from embryos germinated in the dark for 72 hours, infiltrated with isotope, and allowed to germinate further under various conditions. All incubations were in the dark unless indicated otherwise.

same base composition as the rRNA species extracted from ribosomes, yet they have different sedimentation characteristics (*9*). In most instances, in *E. coli* the difference in sedimentation characteristics between "nascent" and "mature" rRNA is most easily shown for the light rRNA. In the case of cotton the consistent difference relates to the heavy rRNA, whereas the separation of "nascent" and "mature" rRNA of the light species is more difficult to demonstrate on a sucrose gradient. The difference in *E. coli* rRNA in sedimentation properties has been ascribed to a difference in secondary structure between rRNA that has not yet become methylated and rRNA that is derived from the breakdown of ribosomes.

Our data concerning the nature of the labeled RNA from this stage of germinating cotton are open to interpretations other than that of its being "nascent" rRNA. The notion that the labeled particle is indeed a ribosomal precursor particle is based first on the fact that it can be fragmented into two molecular species of RNA whose base composition, within experimental error, corresponds to that of the two species of rRNA from cotton; and secondly on the fact that the labeled RNA species derived from the particle have sedimentation properties distinct from those of mature rRNA, a phenomenon reported by a number of workers to apply to "nascent" rRNA from *E. coli* (*9*). In addition, the germinating cotton particle is very similar to the *E. coli* particles formed during inhibition of protein synthesis with respect to its relative position on sucrose-gradient profiles of ribosomal subunits (*10*). There is the possibility that the 45*S* particles of HeLa cells, the 25 to 30*S* particles in *E. coli*, and the germinating cotton particle may indicate an analogous sequence of events in ribosome synthesis in all organisms.

In any explanation for the synthesis and accumulation of this particle by cotton embryos at this stage of germination one must consider that the synthesis of what is here described as "nascent" rRNA is demonstrable only by isotope incorporation, and probably represents a negligible contribution to embryonic development.

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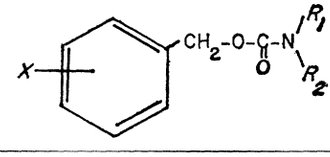
3,4-Dichlorobenzyl Methylcarbamate and Related Compounds as Herbicides

Abstract. *3,4-Dichlorobenzyl methylcarbamate is a selective preemergence herbicide active against both grass and broadleaf weeds. Synthesis of pigment is inhibited in species sensitive to this chemical. Minor structural modifications, such as positioning of the chlorine on the phenyl ring or variations in the* N*-substituent, significantly alter its activity.*

Esters of certain carbanilic acids have been known for many years as regulators of plant growth. Friesen (*1*) was the first to recognize the inhibition of oats and wheat by ethyl carbanilate (phenylurethane). Limited research (*2*) concerning effects of various carbanilates on plant growth continued until the discovery (*3*) of the growth-inhibiting properties of isopropyl carbanilate, IPC. The practical utility of IPC as an herbicide for selective removal of grass weeds from broadleaf crops stimulated additional research that led to the discovery of the herbicidal properties of isopropyl *m*-chlorocarbanilate, CIPC (*4*).

Major efforts have been directed primarily toward the exploration and utilization of carbanilates as herbicides.

Table 1. Herbicidal activities of various benzyl carbamates rated (10 days after treatment) on the following basis: 0, no injury; 1, slight injury, slight reduction in stand, or both; 2, moderate injury, moderate reduction in stand, or both; 3, severe injury, severe reduction in stand, or both; 4, complete kill.



Compound	Substituent			Post-emergence (4 kg/hectare)		Pre-emergence (5 kg/hectare)	
	X	R_1	R_2	Grass*	Broad-leaf†	Grass‡	Broad-leaf§
(A)	H	CH_3	H	0	2	0	0
(B)	2-chloro	CH_3	H	0	2	0	0
(C)	3-chloro	CH_3	H	2	4	4	4
(D)	4-chloro	CH_3	H	2	5	3	6
(E)	2,3-dichloro	CH_3	H	0	2	0	1
(F)	2,4-dichloro	CH_3	H	4	7	0	0
(G)	2,5-dichloro	CH_3	H	0	2	0	0
(H)	2,6-dichloro	CH_3	H	0	2	0	0
(I)	3,4-dichloro	CH_3	H	4	5	7	7
(J)	3,5-dichloro	CH_3	H	2	4	2	2
(K)	3,4-dichloro	H	H	2	6	4	5
(L)	3,4-dichloro	CH_3	CH_3	0	2	2	0
(M)	3,4-dichloro	C_2H_5	H	4	8	1	0
(N)	3,4-dichloro	*i*-C_3H_7	H	2	3	1	1
(O)	3,4-dichloro	*n*-C_4H_9	H	2	3	2	1
(P)	3,4-dichloro	*t*-C_4H_9	H	0	0	0	0
(Q)	3,4-dichloro	allyl	H	2	6	0	0
(R)	3,4-dichloro	phenyl	H	2	4	0	0
(S)	3,4-dichloro	phenyl	CH_3	0	1	0	0
(T)	3,4-dichloro	benzyl	H	0	2	0	0

* Corn, actual rating (0 to 4) × 2. † Tendergreen bean and tomato, total for both. ‡ Perennial rye grass and pearl millet, total for both. § Mustard and pigweed, total for both.