number of such strains, establishes a causal connection between the ethionine resistance and temperature sensitivity of *r-eth-1*.

Of 97 cultures from the aforementioned cross which we tested for mating type, we found four *r-eth-1*-A and two wild type-a recombinants, the remainder being of the parental mating types. Thus the gene in question is on chromosome 1, roughly six crossover units from the mating type locus. Having established the linkage group to which *r-eth-1* belongs, we obtained all the known irreparable mutants which are located on chromosome 1. Of these strains, STL-6-A, b-39-a, *44409-t*-A, *46006-t*-a, and *55701-t*-A, the latter was ethionine-resistant in the usual test system, and we found it to be irreparable on the same complex media that we used to demonstrate this point with *r-eth-1*. Strain STL-6-A grew slowly and with an abnormal morphology on ethionine. The remaining three strains were fully sensitive to ethionine.

The *55701-t* gene is known to be extremely closely linked to mating type, whereas *46006-t* is located about six crossover units to the right of the latter, at or close to the centromere (*6, 7*). Strain b-39 is far distal to mating type on the left arm of chromosome 1 (*8*); *44409-t* and STL-6 are on the right arm, not closely linked to the centromere (*7*).

We crossed *55701-t* to *r-eth-1* and plated random spores on minimal agar (200 spores to a petri dish), subjected them to heat shock, allowed them to germinate for 6 to 8 hours at 24°C, and then incubated the plates for 12 hours at 38° to 40°C. Of a total of 2450 spores examined, 72 temperature-permissive recombinants were observed. The germination rate was 91 percent. If it is assumed that all the inviable spores were mutant, and that the double mutant was temperature-sensitive, these two loci must be about six crossover units apart. We transferred 12 of the presumed wild-type recombinants onto minimal agar. All were similar to wild type in their growth rate at 38° to 40°C and were fully ethionine-sensitive. We found all of them to be of mating type *a*, consistent with the notion that *55701-t* is much more closely linked to mating type than is *r-eth-1*.

Strain *46006-t*-a was similarly crossed to *r-eth-1*-A. Germination of the random ascospores was virtually 100 percent. Of 2000 sporelings, 19 wild type growth centers appeared, corresponding to a map distance of about 1.9 crossover units. Thus, *r-eth-1* lies to the right of mating type and *55701-t*, near the centromere of chromosome 1. All of the wild type recombinants were mating type a, showing that *r-eth-1* is to the left of *46006-t*.

As expected, crosses of *r-eth-1* to STL-6 and to *44409-t* showed relatively weak linkage, with many wild type growth centers. It is rather surprising to find the two ethionine-resistant strains *r-eth-1* and *55701-t* reasonably closely linked, but in positions sufficiently remote that they are not part of the same cistron or probably even of the same operon.

The *r-eth-1* mutant synthesizes excessive quantities of methionine (*9*), and appears to have lost repression control over this pathway. Thus the basis of resistance does not seem to be an activating enzyme mutation, as found by Fangman and Neidhardt (*10*) in the case of a fluorophenylalanine-resistant mutant of *Escherichia coli*, nor is our mutant similar to the ethionine-resistant strains of *Coprinus* described by Lewis (*11*). Numerous cases of drug resistance have been successfully explained on the basis of overproduction of the normal congener of the drug (*12*), and Ames has recently made a useful summary of additional mechanisms by which a microorganism may acquire resistance to an inhibitory analog (*13*). However, it remains to be explained why the loss of control over methionine synthesis should result in an irreparable failure to grow at elevated temperatures. One attractive possibility is that an indispensible macromolecule, perhaps the methionyl transfer RNA, normally functions as the repressor substance for the methionine pathway; in the mutant under study, this may have been so altered that it is temperature-labile and concomitantly fails to be recognized as a repressor.

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Intranuclear Site of Histone Synthesis

Abstract. *The nucleolus is implicated as a site of synthesis of nuclear basic proteins. Some of these newly synthesized proteins derived from the nucleolus have been characterized chemically as histones.*

In higher organisms histones are of considerable importance in chromosome structure and regulation of DNA transcription (*1*) and replication (*2*). Although predominantly associated with the cell nucleus, histones have also been found in cytoplasmic ribosomes (*3*). The extreme heterogeneity of the histones, as revealed by column chromatography (*4*) and electrophoresis (*5*) may reflect a similarly large diversity of function.

We showed previously that the normally concurrent production of histone and DNA (*6*) may be uncoupled by the action of 5-fluorodeoxyuridine which inhibits DNA synthesis while permitting histone synthesis to continue (*7*). We concluded that histone synthesis is not under the direct control of new synthesis of DNA and that the production of the histone molecule may well precede its association with DNA.

We have now investigated the role of subnuclear components in the synthesis of both basic and residual proteins. For the purpose of this report histones are defined according to standard extraction procedures as proteins soluble in acid, precipitated by base and lacking-SH groups as indicated by their solubility in the Mirsky-Pollister reagent (*8*).

Nuclei were prepared from 36-hour-old pea seedlings (*9*) or 5-day-old exponentially dividing cells of tobacco

Table 1. Distribution of radioactivity among subnuclear components of tobacco cells after a 40-second incubation period with C^{14}-labeled amino acids in vivo. Cells (300 g fresh weight) were incubated in 300 ml of medium in the presence of 100 μc C^{14}-labeled algal protein hydrolysate at 27°C for 40 seconds. The reaction was stopped by the addition of 10 volumes of ice-cold grinding medium (*10*), and the cells were removed from the incubation medium by filtration. The cells were fractionated into nuclei (*10*) and subnuclear fractions (*11*) as previously described. Each value represents the average of three experiments.

Sedimentation (10^3 rev/min)	C^{14}-amino acids incorporated (count/min)		
	Histone	Basic non-histone	Residual
Nucleolar fraction			
8*; 14*	64	76	203
Nucleoplasmic fraction			
25*; 40†	22	32	229

* Spinco rotor No. 25. † Rotor No. 40.

cell cultures (*10*), fractionated into subnuclear components as described earlier (*11*). The basic proteins were extracted from the subnuclear particles with 0.2*N* HCl in the cold (*12*), precipitated by the addition of 1/10 volume of concentrated ammonia and 2 volumes of ice-cold ethanol and fractionated further into histones and basic, non-histone proteins (*8*).

After a 40-second pulse with amino acids labeled with C^{14} the nucleolus incorporated more radioactivity than the nucleoplasm. In both systems, tobacco cells (Table 1) and isolated pea nuclei (Table 2), radioactivity was found in histone and nonhistone basic proteins and to an even larger extent in the residual fraction of the nucleolus, while the corresponding nucleoplasmic fractions showed less activity. After longer periods of incubation (90 minutes) the extranucleolar protein fractions rapidly accumulated radioactivity and incorporated larger amounts of C^{14} than those of the nucleolar fraction (Table 3).

Table 2. Distribution of incorporated leucine-H^3 into different subnuclear components of pea nuclei after a 3-minute incubation period in vitro. Isolated nuclei were incubated with 50 μc/ml of DL-leucine-H^3 (5.4 c/m*M*) at 37°C. The incubated nuclei were purified by sedimentation through a sucrose density gradient (2.0 to 0.8*M*) and fractionated into subnuclear constituents (*11*). The extracted protein fractions were washed repeatedly (*13*) and counted. Each value represents the average of four experiments.

Fraction (*11*)	Leucine-H^3 incorporated (count/min)		
	Histone	Basic non-histone	Residual
Nucleolus	110	260	4,180
DNA-rich nucleoplasm	60	90	1,050
RNA-rich nucleoplasm	0	10	310
Non-particulate nucleoplasm		360*	

* Unfractionated.

For incubation periods such as those used, there is no measurable turn-over of nuclear proteins (*7*). That exchange or transfer of protein between nucleus and cytoplasm does not enter into the results obtained from whole cells has been indicated by "pulse-chase" experiments. Tobacco cells were "pulse labeled" by incubation for a short period of 10 minutes with C^{14}-labeled lysine. The C^{14}-labeled amino acid was then "chased" by transfering the cells to fresh culture medium containing a 1000-fold excess of unlabeled lysine. As a result of this chase further incorporation of the C^{14} activity into proteins of the cell cultures was virtually abolished. It may be seen from Fig. 1 that during this chase there was no significant exchange of radioactivity between nucleus and cytoplasm. It is concluded that cytoplasmic proteins labeled during the initial 10-minute pulse did not migrate to the nucleus to any measurable extent.

The fact that isolated nuclei (Table 2) or nucleus-free nucleolar and chromatin preparations (*13*) exhibited intranuclear labeling patterns similar to those of nuclei of whole cells (Table 1) lends further support to the view that the cytoplasm contributes but little, if any, to the nucleus in the time interval studied (approximately 5 percent of the generation time of the tobacco cells).

The high C^{14}-activity of the nucleolus after a very short period of incubation is in contrast to the predominance of C^{14} label in the nucleoplasmic fraction after a prolonged incubation (Tables 1 and 3). Thus, in the case of histones there was a shift in the ratio of radioactivity (nucleolus to nucleoplasm) from 2.5 : 1 initially to 1 : 2 at later times.

Two possibilities may be considered for the cytological interpretation of the redistribution of the radioactivity. (i) Histones are synthesized within the nucleolus and after being released from the sites of synthesis appear ultimately in the nucleoplasmic fractions because of the partial mechanical abrasion of the nucleolar periphery (*14*) during the isolation of the subnuclear components.

Table 3. Distribution of radioactivity between nucleolus and nucleoplasm after incubation with C^{14}-labeled amino acids in vivo for 90 minutes (tobacco cells). Conditions as in Table 1, but only 25 μc of C^{14}-algal protein hydrolysate were used. Each value represents average of two experiments.

Sedimentation (10^3 rev/min)	C^{14} amino acids incorporated (count/min)		
	Histone	Basic non-histone	Residual
Nucleolar fraction			
8*; 14*	171	138	1,885
Nucleoplasmic fraction			
25*; 40†	305	216	3,536

* Spinco rotor No. 25. † Rotor No. 40.

(ii) Alternatively, the shifts indicate that histones are synthesized in the nucleolus and that there is a genuine migration of these proteins to the (possibly contiguous) extranucleolar nucleoplasm.

Similar arguments can be applied to the residual proteins, which have an amino acid composition virtually identical with that of ribosomal proteins

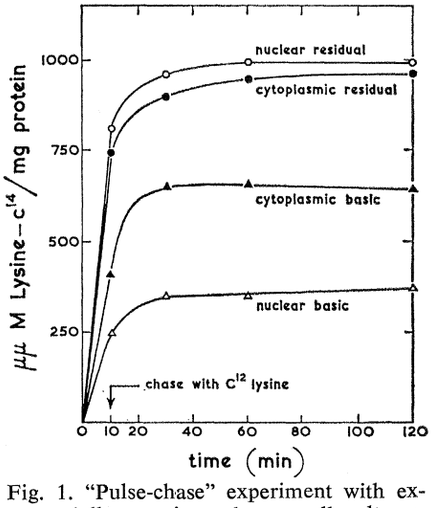

Fig. 1. "Pulse-chase" experiment with exponentially growing tobacco cell cultures. Conditions were the same as described in Table 1, but 1 μc/ml of DL-lysine-C^{14} was used. After incubation with the labeled lysine for 10 minutes at 27°C the cell suspension was filtered and resuspended in culture medium containing a 1000-fold excess of unlabeled DL-lysine and the incubation continued. At intervals equal portions were withdrawn, fractionated into nuclei (*10*) and nuclear supernatant (cytoplasmic fraction) and the radioactivity determined in the protein fractions. The results show that the labeled lysine was incorporated very rapidly in all fractions. After addition of unlabeled lysine the C^{14}-activity of all fractions leveled off and there was no indication of a rapid exchange between the cytoplasmic and nuclear proteins which can be extracted with acid.

(*15*). The shifts in the ratios observed for this protein fraction are consistent with the idea that ribosomal structural proteins are synthesized in the nucleolus and form complexes with newly synthesized ribosomal RNA (*16*) in the nucleolar periphery (first alternative) or within the chromatin (second alternative).

As yet we cannot discriminate between these alternatives. The results we obtained using short term incubation periods, however, clearly show that the nucleolus plays an important role in the protein synthesis of pea and tobacco nuclei. In particular, the nucleolus synthesizes an appreciable portion of the nuclear basic proteins. Some of these proteins have been characterized as histones.

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Culture of Embryonic Cells of Drosophila melanogaster in vitro

Abstract. *Embryonic cells isolated from eggs of* Drosophila melanogaster *have been cultured continuously in a new medium. Generation time for cell division is 30 hours. Chromosome number remains constant for at least 10 days. Cells from embryos of the mutant* maroon-like *grow at the same rate as those from wild-type embryos, but cells from* rosy-2 *grow slower and at a lower optimum temperature.*

The potential value of the techniques of cell culture for studies in somatic cell genetics, in the problems of differentiation, and in virology is widely recognized. Full realization of these potentialities, however, has been limited by the genetic and cytological complexities of the organisms where true cell culture has been possible. *Drosophila melanogaster,* the classical object of genetic study, would seem to be ideally suited for this purpose, but successful cultivation of its cells in vitro has not previously been achieved. The successful culture of insect cells by any method has been achieved only rarely (*1*). In this paper we report the development of a new culture medium and techniques which have resulted in the successful cultivation of embryonic cells from various developmental stages of *D. melanogaster.*

Embryonic cells were obtained from eggs of a wild stock (Oregon R-EL2) and from two eye-color mutants, *rosy-2* (*ry*^{2}) and *maroon-like* (*ma-l*). Eggs of highly uniform developmental stages were collected by use of the ovitron described by Yoon and Fox (*2*). Eggs of lesser uniformity were collected after oviposition on filter papers moistened with a suspension of standard corn meal-agar-molasses and killed yeast. One thousand to three thousand eggs collected by these methods were immersed for 2 minutes in 3 percent sodium hypochlorite. They were then rinsed with twice-distilled water and sterilized for 15 minutes in 0.05 percent $HgCl_2$ in 70 percent ethyl alcohol. All subsequent steps were carried out in a sterile-transfer chamber. The sterilized eggs were rinsed three times with H-5 culture medium (Table 1), and were homogenized lightly and slowly with 1 ml of H-5 medium in a 2-ml glass homogenizer.

By this method the embryos were dissociated into single cells with minimum damage. The suspension was then passed through a sintered glass filter (maximum pore size, 170 to 220 μ) for removal of the broken vitelline membranes, and were mixed by means of a pipette with 15 ml of H-5 medium supplemented with 10 percent newborn calf serum.

For growth, 5-ml portions of the cell suspension were transferred to T-30 culture bottles and cultured without shaking in an incubator aerated with normal air. For the determination of cell multiplication, 1-ml portions of the cell suspension were distributed into 12 by 100 mm test tubes, and the tubes were incubated at an angle of 5 degrees to the horizontal. After 0, 2, 4, and 7 days of cultivation, the numbers of cells were determined with a hemocytometer of the Bürker type. The average of three culture tubes

Table 1. Composition of medium H-5.

Constituents	Amount (mg/1000 ml)
Salts	
$NaH_2PO_4 \cdot 2H_2O$	200
$NaHCO_3$	350
KCl	200
$CaCl_2 \cdot 2H_2O$	20
$MgCl_2 \cdot 6H_2O$	100
NaCl	7,000
Sugars	
Glucose	13,800
Sucrose	13,800
Antibiotics	
Penicillin G (sodium salt)	30
Streptomycin sulfate	100
Peptides	
Glutathione	5
Amino acids	
Lactalbumin hydrolysate	17,500
L-tryptophane	100
L-cysteine hydrochloride	25
Vitamins and nucleotides	
Yeast extract	1,500
Ascorbic acid	100
Niacinamide	0.1
Nicotinamide-adenine dinucleotide	5
Organic acids	
Sodium acetate	25
Malic acid	670
Succinic acid	60
pH Indicator	
Phenol red	10

*p*H adjusted to 6.5 with 1*N* KOH. Sterilization by passage through a Seitz filter.