SYNTHESIS OF MESSENGER-LIKE RIBONUCLEIC ACID AND PROTEIN DURING MEIOSIS IN ISOLATED CELLS OF *TRILLIUM ERECTUM*

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ABSTRACT

The synthesis of RNA and protein by cultures of isolated microsporocytes has been demonstrated. The variation in capacities of such cultures to perform syntheses is a function of meiotic stage and parallels the pattern of changes observed for microsporocytes *in situ*. A principal feature of this pattern is the induction of syntheses during pachytene and diplotene, stages at which the chromosomes are partly contracted. By use of Actinomycin D, chloramphenicol, pulse-labeling with P^{32}-phosphate, and nucleotide analyses of RNA digests, part of the RNA synthesized has been shown to correspond to messenger RNA. Analysis of reaction rates and the overlappings of protein and RNA synthesis indicates that the spread of cytological events in *Trillium* is not purely a function of the low temperature at which it occurs but, presumably, arises from a complement of regulatory devices which govern the periodic onset of reactions within the cells. The main conclusion drawn from the whole of these studies is that the sequence of morphological changes associated with chromosome contraction and movement during meiosis is accompanied by a set of gene transcriptions. Although comparatively few genes are presumed to be active during meiosis, the action of such genes may be essential to a translation of some of the information embodying the meiotic sequence which has been stored in the genome in the course of evolution.

INTRODUCTION

This report is concerned with the problem of macromolecular syntheses during meiosis. In a study of anthers cultured "*in vitro*," evidence was obtained which pointed to the occurrence of such syntheses during late meiosis (3). The observations had potentially important physiological implications but also clear experimental limitations. From the physiological standpoint, synthesis of macromolecules at a time when chromosomes were partly contracted had direct bearing on the possibility that some of the genes concerned with meiosis acted during the cycle and not by discharging products in anticipation of the cycle. From the experimental standpoint, the reported observations were limited in two ways: (1) Evidence for synthesis was obtained by exposing intact anthers to labeled precursors, and isolating the meiotic cells (microsporocytes) for analysis. Since the level of isotope incorporation by the microsporocytes was much below that of the adjacent somatic tissues, contamination could not be ruled out; (2) Meiotic upsets induced by reagents inhibitory to RNA or protein synthesis could not be unequivocally attributed to interference with macromolecular syntheses in the meiotic cells either because of the possibility that these reagents acted through the adjacent somatic tissues or because similar upsets by other types of reagents had been reported (13).

To obviate the limitations imposed by studies

of intact anthers, experiments were designed in which the meiotic cells were isolated from their host organ and their capacity to synthesize RNA and protein "*in vitro*" was measured as a function of meiotic stage. In the results which follow, it will be shown that the patterns of synthesis under *in vitro* conditions are much the same as those under "*in situ*" conditions and that part of the RNA synthesized during different meiotic intervals has the earmarks of "messenger RNA."

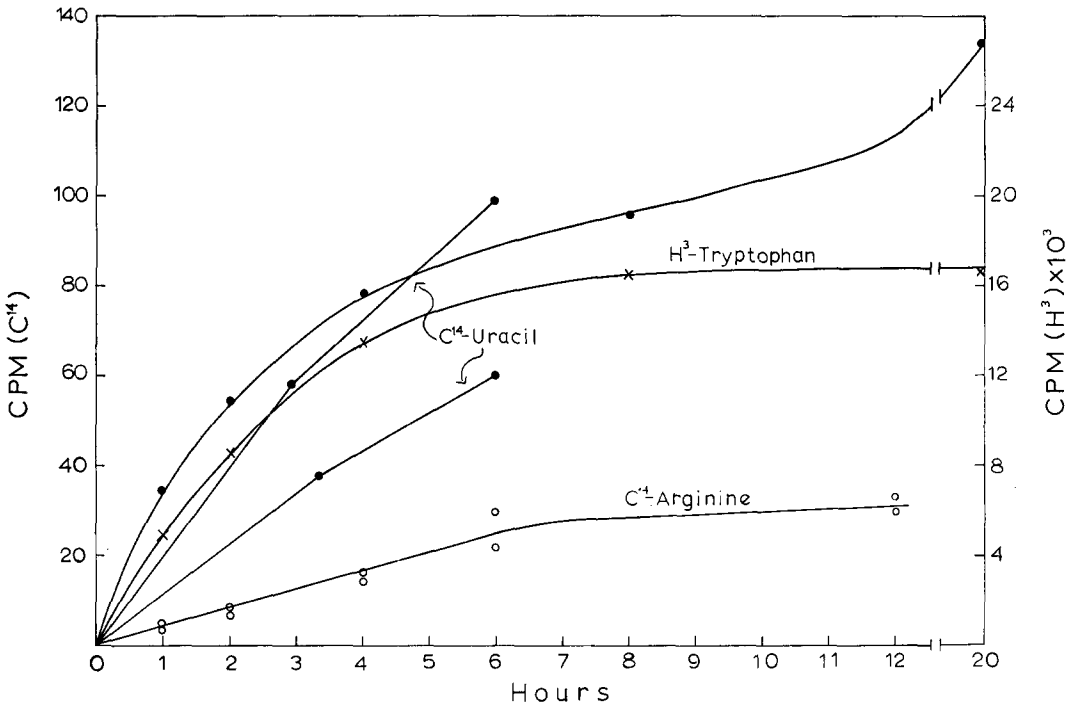

FIGURE 1 Time course of incorporation of labeled precursors into protein and RNA of isolated microsporocytes or microspores under "standard conditions." The curves for uracil and tryptophan were obtained with cells in leptotene. Arginine incorporation into protein was followed with early microspores. It will be seen later that the incorporation of arginine is very much below that of leucine, tryptophan, or glycine throughout the meiotic cycle but rises sharply at an interval close to that of DNA synthesis. Although activities are represented as CPM for cells derived from 10 anthers (see Methods), it is to be understood that in most experiments at least 50 anthers were used for each incubation.

METHODS

The general handling of *Trillium* plants has been described previously (3). Microsporocytes were mechanically extruded from anthers and suspended in standard incubation medium. The cells were washed free of contaminants by two sedimentations at 1500 *g* for 8 minutes. Microscopic examination of the suspension revealed very few contaminants; it will be shown later, however, that the contaminants had little effect on measured synthetic rates.

Each ml of standard incubation medium contained the following components: (in micromoles) sucrose, 350; glucose, 110; Tris(hydroxymethyl)aminomethane or phosphate buffer (pH 7.0), 10; $CaCl_2$, 1.5; $MgCl_2$, 1.5; (in millimicromoles) adenosine, 37.4; guanosine, 34.5; cytidine, 41.2; uridine, 41.0; deoxyadenosine, 39.8; deoxyguanosine, 37.4; deoxycytidine, 44.0 thymidine, 41.2; L-lysine, 86; L-arginine, 37; DL-methionine, 87; DL-threonine, 109; DL-valine, 111; DL-isoleucine, 79, DL-phenylalanine, 31; L-leucine, 119; L-tryptophan, 20; L-glutamine, 103; L-aspartate, 45; L-proline, 43; L-cysteine, 10; glycine, 133. final pH of the medium was 7.0. Aseptic conditions were maintained as previously described (3).

In each assay, the microsporocytes isolated from 10 to 50 anthers were suspended in 30 μl of incubation medium from which the component corresponding to the isotope used was omitted. The following

amounts of isotope (in microcuries) were used in a suspension: C^{14}-uracil, 0.084 (74,000 CPM); C^{14} leucine, 0.053 (47,000 CPM); C^{14}-arginine, 0.032 (28,158 CPM); H^3-tryptophan, 0.5 (110,000 CPM); C^{14}-glycine, 1.92 (1,693,000 CPM). In standard assays, the cells were incubated at 4°C for 4 hours with shaking at 45 strokes per minute. Unless otherwise indicated, activities are expressed as the amount of isotope incorporated into RNA or protein per microsporocytes from ten anthers over the 4-hour period. Chemical methods of fractionation were the same as those described previously (3). Certain details of procedure in relation to RNA composition will be discussed later.

RESULTS

A primary requirement for these studies was the availability of a medium favorable to cell viability. The choice of such a medium is made difficult by the distinctive organic environment in which microsporocytes and microspores develop. Simple media, such as "Hoagland's" (3), which are satisfactory for many physiological studies of plant tissues, cause an immediate coagulation of the protoplasts of the germinal cells. Indeed, there was no attempt in these studies to discover a medium which would permit the cells to undergo the full cycle of meiosis and mitosis, a process extending for approximately 3 months. For present purposes, it was sufficient if the biochemical capacities of the isolated cells could be tested over comparatively brief intervals of time, 3 to 4 hours. The criteria used in choosing a suitable medium were as follows: (1) Appearance of unstained cells. Coagulative effects, especially nuclear contraction, are easily spotted, and where such were seen at the end of the incubation period, the medium was considered unfavorable. (2) Appearance of stained cells. If nuclei or chromosomes appeared pycnotic after staining with propionocarmine, the medium was not used. (3) The course of incorporation of isotopically labeled compounds. A 4-hour period of incubation at 4°C was chosen somewhat arbitrarily as the standard test procedure. The medium was considered satisfactory if the rate of incorporation during this interval was approximately linear and if appreciable incorporation followed the 4-hour period. The first two criteria were satisfactorily met in the medium described under Methods; the third criterion was only partially fulfilled. This is evident in Fig. 1.

Since a linear rate of incorporation was not maintained over prolonged intervals, microsporocytes were tested for their ability to incorporate isotope into RNA after being incubated without isotope for 2 to 3 hour intervals. It may be seen from Table I that rates of incorporation are much the same for the period tested, whether or not the cells were preincubated. Since the standard interval of assay was 4 hours, it was reasonable to conclude that rate of uptake was not appreciably affected by the duration of the test period.

TABLE I

Effect of Preincubation on Capacity of Isolated Microsporocytes to Synthesize RNA

Exp. No.	Preincubation time	CPM P^{32}-RNA	CPM C^{14}-uracil RNA
	hrs		
1	0	—	30
	2	—	29
2	0	2320	—
	2	2050	—
3	0	—	44
	2	—	48
4	0	—	86
	3	—	86
5	0	—	60
	3	—	62

In each experiment a suspension of microsporocytes collected from 60 to 100 anthers was divided equally, one part being immediately exposed to isotope for 1 hour at 4°C, the other being preincubated for the time indicated. Activities are expressed as total counts for microsporocytes derived from 10 anthers during one hour. Stages here tested were leptotene-zygotene. In experiment No. 2, 0.05 μc of P_{32}/ml of incubation medium was used.

The results obtained, though not ideal, appeared to be sufficient for the purpose of these experiments. The conclusion was reinforced by the fact that the amount of isotope taken up by the cells *in vitro* (3) was similar to that obtained in studies of the cells *in situ*, a fact which became evident when the total cycle was studied. Moreover, if an unphysiological temperature such as 35°C was used for incubation of *Trillium* microsporocytes, none of the listed criteria were met.

The conditions of the experiments were thus favorable, if not ideal. It might be supposed, however, that the incubation medium, though favorable, was lacking essential ingredients which were present in the normal organic environment of the cells. The possibility that such a lack might seriously impair the synthetic capacities of the cells was tested with labeled arginine since its incorporation into protein was consistently low. One group of cells together with the surrounding viscous fluid was mechanically separated from 25 anthers and 30 μl of incubation medium added to the droplet of cells; a second group of similar size was collected but washed in the standard fashion prior to addition of medium. The counts in the extracted protein of each of the groups after 4 hours' incubation were 39.3 and 45.1, respectively. Thus, at least with respect to arginine, the components of the organic fluid had no enhancing effect on protein synthesis; if anything, they appeared to have a depressing effect which was probably a consequence of isotope dilution.

RNA Synthesis as a Function of Meiotic Development

The distinctive and special physiological features of meiosis in *Trillium* are common knowledge but, to avoid the risk of misunderstanding, a few of the salient features will be recapitulated. The plants are harvested in the fall and reach the laboratory in a dormant condition. This dormancy is partly broken on exposing the plants to near-freezing temperatures (8). The break in dormancy is restricted to the reproductive tissues; under both laboratory and natural conditions the single cycles of microsporocyte meiosis and microspore mitosis are completed at temperatures close to 0°C. The precise stage at which the microsporocytes become dormant is unclear, but when examined in the laboratory prior to cold exposure the meiotic cells are fully differentiated and, to our knowledge, have already undergone DNA synthesis. Since leptotene and zygotene phases of the cycle have a more or less uniform cytological complexion, the sole developmental parameter available is the time of cold exposure. In a popuulation of plants, meiosis is loosely synchronized and, as a rough measure of development, the time elapsed since cold exposure is adequate. Once the cells reach pachytene, cytological appearance is the marker of choice. Synchrony within and between the anthers is best at earlier meiotic stages and as the interval of time per stage shortens the spread in development increases. For the biochemical tests performed here it was virtually impossible to select a sufficiently large number of cells at identical cytological stages of development from the first metaphase of meiosis on. In the figures which follow, the points lying between these stages represent an approximation of the proportions of adjacent stages in the suspension tested.

The capacity of isolated microsporocytes to incorporate labeled uracil into RNA as a function of meiotic stage is shown in Fig. 2. The first feature to be noted is that between about 20 to 35 days following cold exposure there is a marked but transient increase in the capacity of the cells to synthesize RNA. Whether the bimodality of the curve is a real or fortuitous result is uncertain because of the lack of developmental criteria other than time. The second feature of interest is the renewal of synthetic capacity at a much later stage in meiotic development. At diplotene, chromosome thickening is evident. Further contraction of the chromosomes during diakinesis and metaphase I is associated with a drop in synthetic capacity. Although there is no appreciable interphase between the first and second meiotic divisions, it is clear that the capacity to synthesize RNA is again increased prior to the termination of the cycle. Once the cells reach the tetrad stage, synthetic capacity increases markedly and by microspore interphase the magnitude is of a much higher order than that prevailing during meiosis. The characteristics of interphase development have been dealt with elsewhere and will not be discussed here (4).

A comparison of the results obtained in these studies with those previously reported for cultured anthers (3) indicates that the fluctuations in capacity of isolated microsporocytes to synthesize RNA reflect the fluctuations occurring with respect to *in situ* synthesis. The two curves are not strictly comparable (see Fig. 2) since, in the case of cultured anthers, accumulation of label was measured over long periods of time. It will be seen, however, that where comparable measurements have been made (Fig. 3) the parallel is complete. On the basis of these results it would appear therefore, that meiosis is associated with a regulated RNA synthesis, even during intervals when the chromosomes have undergone extensive morphological alteration.

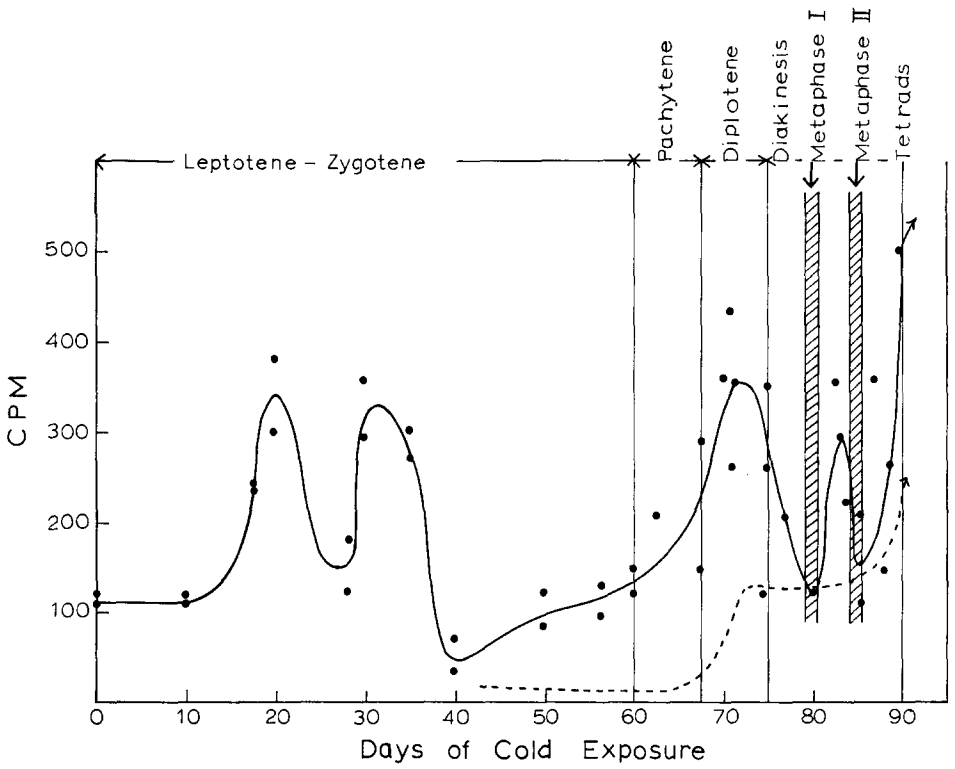

FIGURE 2 Incorporation of C^{14}-uracil into RNA of isolated microsporocytes. At day 0 (October), plants were put into storage at 1°C. Each point in the graph represents a single experiment in which microsporocytes isolated from 2 to 5 buds were used. The larger number was used at stages of low activity. All values are expressed in terms of the microsporocytes obtained from 10 anthers. The total protein content of such a pool of cells is approximately 0.8 mg, and the RNA approximately 0.003 mg. Little cytological change is evident during the first 50 to 60 days of cold storage. Once pachytene was reached, subsequent development was followed cytologically. The dotted line represents the accumulation of C^{14}-uracil into RNA of microsporocytes of intact anthers continuously exposed to label (3). Conditions of incubation are described under Methods.

Protein Synthesis as a Function of Meiotic Development

Three labeled precursors—leucine, glycine, and tryptophan—give much the same result. Once pachytene is reached there is a sharp rise in the capacity of microsporocytes to synthesize protein. Moreover, there appears to be a distinctive pattern of changes. With each of the three compounds tested a bimodal curve was obtained, a curve very similar to that obtained for cultured anthers (Fig. 3). There is a transient increase in synthetic capacity 20 to 30 days following cold exposure, but it is smaller in magnitude than that occurring during stages of chromosome contrac tion. In this respect, the capacity of the microsporocytes to synthesize protein differs from their capacity to synthesize RNA, but in other respects the patterns are similar though not identical. Thus, inasmuch as the capacities of isolated microsporocytes match their performance *in situ*, it is reasonable to conclude that one facet of meiotic regulation lies in the controlled synthesis of macromolecular components during the process. It is of interest in this connection that arginine incorporation, which might be taken as an indicator of histone synthesis, is almost negligible throughout meiosis. Only after microspores have formed and interphase development has begun does arginine incorporation become quantitatively significant.

The technique of measuring protein synthesis in

isolated microsporocytes presented an opportunity for testing the possibility of contamination by somatic tissues. In an earlier report (3) the ticklishness of this problem was discussed. The metabolic activities of the somatic tissues in intact anthers are much higher than those of the microsporocytes, and although the evidence against *in situ* labeling of microsporocytes' being an artefact of courteous. Although the somatic tissues constitute approximately ten times the mass of the microsporocytes, *their total* uptake was on the average less than that of the microsporocytes and remained constant through all the stages of meiosis (Fig. 4). In isolated cell cultures, therefore, the activity of the somatic cells relative to the germinal ones were negligible on a per unit protein basis. Since

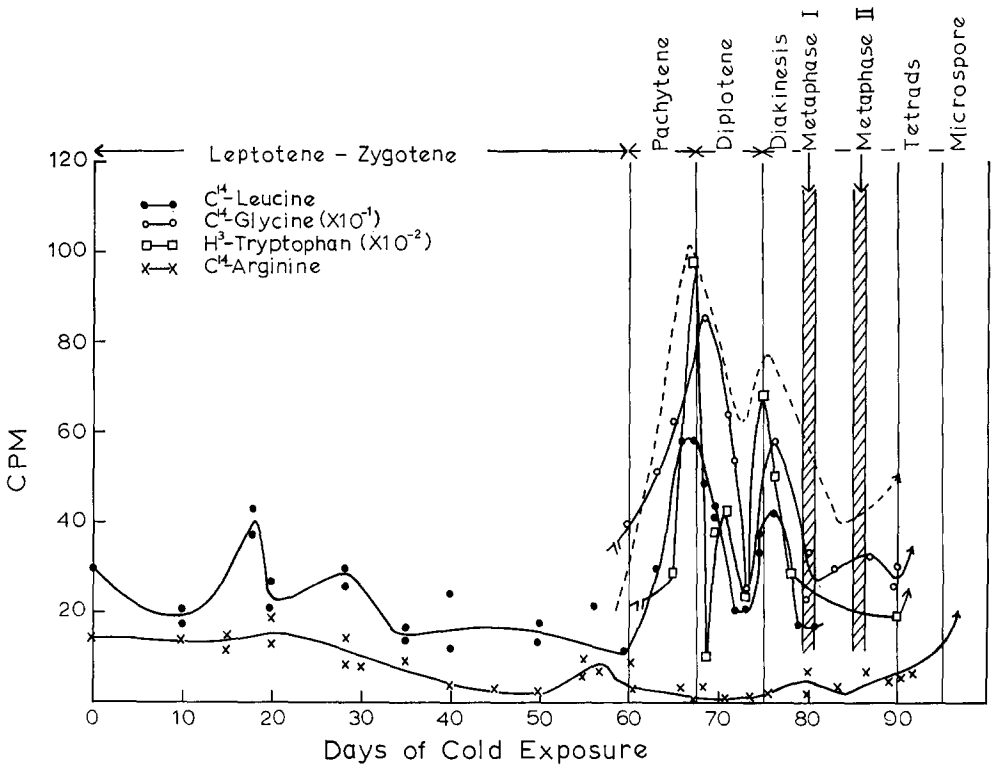

FIGURE 3 Incorporation of labeled amino-acids into proteins of isolated microsporocytes at different stages of meiotic cycle. The dotted line represents the pattern of C^{14}-leucine incorporation by microsporocytes *in situ* (3). Other information may be found in legend to Fig. 2 and under Methods.

osmatic contamination appeared reasonable, it was not conclusive. There was no way of labeling somatic tissues independently and measuring the amount of label which might have been transferred to the apparently clean microsporocyte preparation. In the case of *in vitro* incubations the test was simple. After extruding the microsporocytes the somatic tissues were either loosely homogenized or cut into small fragments; homogenization yielded about 50 per cent intact cells, and cutting a small percentage of broken ones. Both these preparations were tested under the same conditions as the microsporocytes. Nature was microscopic examination of microsporocyte suspensions showed very little contamination, the observed incorporations by microsporocytes cannot be attributed to the somatic tissues. The conclusion that protein synthesis occurs during meiosis appears to be sound, even though the level is considerably lower than that occurring during interphase periods.

Developmental Intervals at the Microscopic and Molecular Levels

In connection with the patterns just described, a question arises which has general significance

and does not bear specifically on meiosis. What is the basis for the prolonged duration of meiotic events in *Trillium* microsporocytes? A meiotic cycle which extends for 90 days is not commonplace. Is it then simply attributable to the low temperature at which meiosis occurs? If so, the difference between logarithmically growing bacteria, for example, and developing microsporocytes could be explained simply by differences in molecular reaction rates. The question obviously implies much more than is encompassed by this example, but we were interested in clarifying a single point: the bearing of molecular reaction rates on the spread of cytological events. With respect to individual enzyme reactions it was shown earlier that thymidine kinase activity in disrupted microspores varied, in the expected way, with the temperature of the reaction mixture (2). A very different relationship between temperature and activity was found, however, when the incorporation of arginine into protein by intact microsporocytes was followed. The relative rates at 2°, 15°, and 25°C were 7.2, 8.1, and 7.4, respectively. Clearly, the intact cell imposed constraints upon the over-all rate of the complex of reactions essential to protein synthesis and these constraints were not lifted by the simple device of raising the temperature.

To push the study further, we sought to establish whether the accepted sequence of RNA-protein synthesis was temporally extended in much the same way as the cytological sequence. It was inviting, at least, to suppose that the rapid sequence of component reactions of protein synthesis in microorganisms was matched by a prolonged and easily dissected sequence in the microsporocytes. A comparison of the curves in Figs. 2 and 3 was not very encouraging; intervals of protein and RNA synthesis overlap. The possibility, nevertheless, remained that identification of developmental stage was not precise enough to allow a direct comparison between different groups of microsporocyte suspensions. To obviate this, a series of double-labeling experiments was

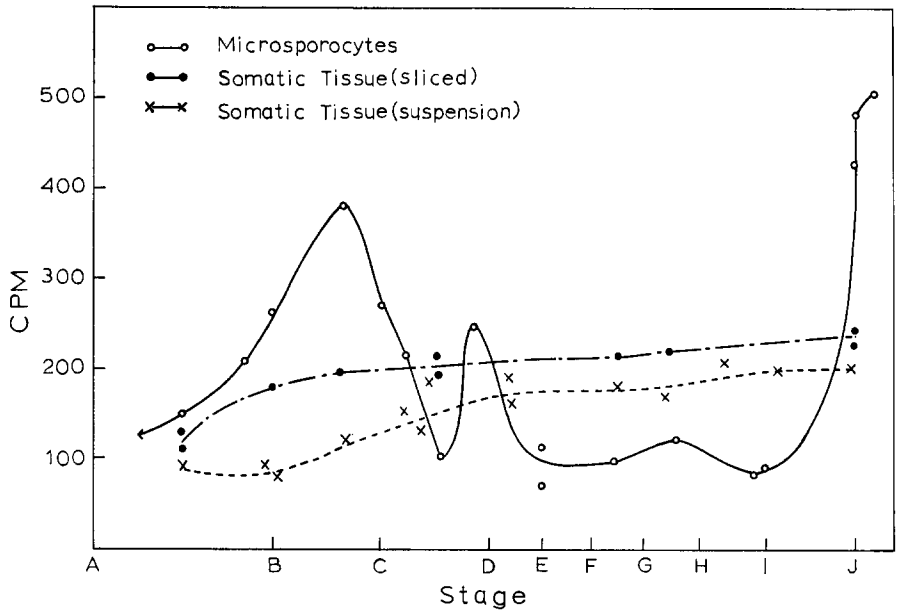

FIGURE 4 A comparison of C^{14}-glycine incorporation by somatic tissues and microsporocytes, respectively, under *in vitro* conditions. Values for microsporocytes are based on cells derived from five anthers. Each point in the curves for somatic tissues represents total incorporation of six anthers per unit protein. The microsporocytes are therefore approximately twelve times as active as the somatic tissues. *Stages: A*, Leptotene-Zygotene; *B*, Pachytene; *C*, Diplotene; *D*, Diakinesis; *E*, Metaphase I; *F*, Anaphase; *G*, Metaphase II; *H*, Anaphase II; *I*, Tetrads; *J*, Early microspores. Other comments in text.

conducted in which isolated microsporocytes were incubated in the presence of C^{14}-uracil and H^3-tryptophan. If an appreciable interval of time existed between RNA and protein synthesis, then striking differences in relative rates of synthesis should have been obtained between groups of microsporocytes at different stages. At the extreme, one might even expect to find stages at which only one of the two macromolecular types is synthesized. The results, however, pointed in a different direction (Fig. 5). There were progressive changes in the ratios of RNA/protein synthesis and, on the whole, the ratio decreased as protein synthesis increased and vice versa; overlapping of activities was, nevertheless, the principal feature of the results. The separated intervals of protein synthesis, therefore, could not be accounted for by an intervening sluggish synthesis of RNA. We interpret these results to signify that the extended nature of the meiotic cycle is a consequence of a regulatory system which is distinct from the chemical events regulated and the rates at which these events occur. The prevalence of a similar relationship during microspore interphase has been documented more specifically elsewhere (2).

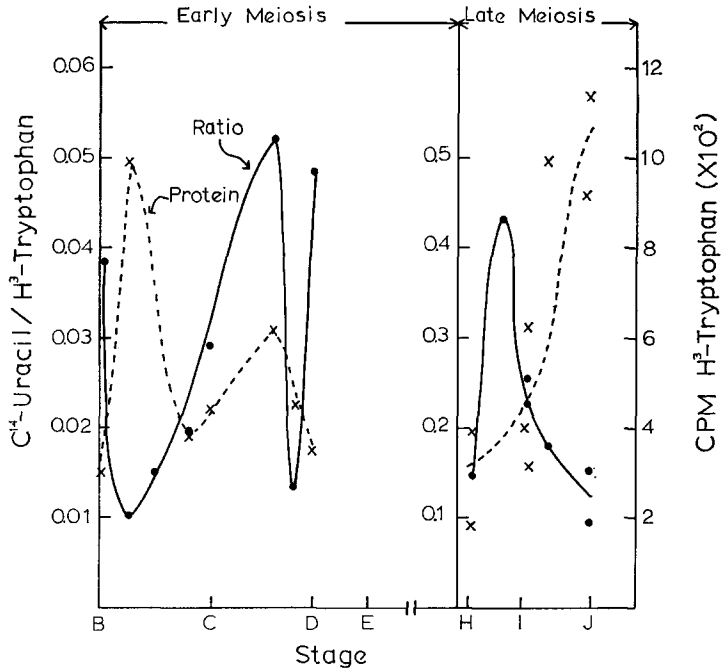

FIGURE 5 Ratio of incorporation of C^{14}-uracil and H^3-tryptophan into RNA and protein, respectively. The two isotopes were administered simultaneously to isolated microsporocytes at stages indicated. A scintillation counter was used to discriminate between the two isotopes. Dotted line represents activity in proteins of microsporocytes from five anthers. Symbols for stages as under Fig. 4. Assay conditions are the same as those described under Methods, except for the presence of two labeled compounds in the medium.

Relationship Between RNA and Protein Synthesis

Although the results of double-labeling experiments pointed to a close temporal relationship between protein and RNA synthesis, additional evidence was sought, circumstantial though it might be, with respect to the generally accepted causal relationship presumed to exist between them. For this purpose two inhibitors were used: Actinomycin D and chloramphenicol. It was taken for granted that these inhibitors would act on a limited number of syntheses because the time of exposure and measurement represented an extremely small interval of the cellular cycle and also because the period of active meiosis was generally marked by a low level of metabolic activity. The questions to which answers were

sought were as follows: (1) Did Actinomycin D and chloramphenicol inhibit RNA synthesis and protein synthesis respectively? On the basis of current interpretations of the action of these inhibitors (1, 7), it was expected that Actinomycin would inhibit RNA synthesis if the latter were specified by DNA and that chloramphenicol would inhibit protein synthesis if the latter were mediated by the RNA-ribosomal complex. (2) Did each of the inhibitors affect one process exclusively? On the assumption that all the protein formed in the microsporocytes was derived *via* RNA, it was expected that the more labile the essential RNA, the more marked would be the effect of RNA inhibition on protein synthesis, and conversely, the greater the stability of the required RNA inhibition on protein synthesis. A similar set of alternatives would be expected in the case of chloramphenicol action if it is presumed that the principal effect of the agent is to block the effective coupling between messenger RNA and ribosome. The results of these experiments are summarized in Table II. With respect to question 1, the answer is reasonably clear. Concentrations of actinomycin as low as 0.5 μg/ml cause maximal inhibition of RNA synthesis. Presumably, 40 per cent of the RNA formed is not susceptible to Actinomycin D; if the claimed specificity and effectiveness of the drug is correct, then such RNA is not derived from DNA. It is impossible, of course, to tell from these studies just what fraction of the total this RNA represents since relative turnover rates of these fractions as well as their concentrations would have to be known. The mixed effectiveness of chloramphenicol on protein synthesis has already been reported (3). If the presumed locus of chloramphenicol action is correct, then the results cannot distinguish between the possibility that two pathways of protein synthesis exist or that activated ribosomal units are unaffected. With respect to question 2, the results are equivocal. Neither inhibitor affects a single process exclusively; each inhibits preferentially in the expected direction. The single conclusion which may be drawn is that the overlapping found in the double-labeling experiments is consistent with the results obtained in these experiments. However, the most important pointer provided by the data is that part of protein synthesis during meiosis follows a pattern similar to that expected if genes are actively specifying the proteins formed. This interpretation lent itself to a further experiment—the base composition of the RNA synthesized.

TABLE II

Effects of Actinomycin D and Chloramphenicol on RNA and Protein Synthesis in Isolated Microsporocytes

Inhibitor	Conc.	RNA inhibition	Protein inhibition	RNA/Protein
	μg/ml	*per cent*	*per cent*	
Actinomycin D	25	61	73	0.84
	5	57	45	1.27
	1.0	61	44	1.39
	0.5	62	46	1.35
	0.1	45	25	1.80
	0.02	10	9	1.11
Chloramphenicol	100	38	53	0.72
	50	14	35	0.40
	10	3	6	0.50

Each assay was made with microsporocytes isolated from 10 anthers. The suspensions of meiotic cells were preincubated with inhibitor for 60 minutes at 4°C; controls were similarly preincubated but without inhibitors. At the end of the preincubation period, 0.084 μc of C^{14}-uracil and 0.5 μc of H^3-tryptophan were added to each tube. Cells used in these experiments were at various stages of meiosis between pachytene and telophase II.

Nucleotide Composition of Pulse-Labeled RNA

Of the various experimental criteria now used to identify messenger RNA (9), the most conven-

TABLE III

Nucleotide Composition of Pulse-Labeled RNA in Microsporocytes and Microspores of Trillium and Iris

Plant	Stage	Inhibitor	Inhibition	Fraction	A	G	C	U	$\frac{C+U}{A+G}$	AU
			per cent							*per cent*
Trillium	Pachytene to	—	—	Nucl.	28.1	20.4	21.4	30.1	1.06	58.2
	Diakinesis		—	Cyt.	24.6	30.6	24.3	20.4	0.81	45.0
	Pachytene to	Actinomycin D	68	Nucl.	25.2	27.9	17.8	29.2	0.89	54.4
	Diakinesis		84	Cyt.	27.6	33.3	24.3	15.5	0.64	43.1
	Tetrads to	—		Nucl.	30.2	19.8	19.8	30.2	1.00	60.4
	Microspores	—		Cyt.	26.2	30.6	23.3	19.9	0.76	46.1
Iris	Pachytene to	—		Nucl.	29.1	22.1	18.4	30.4	0.95	59.5
	Diplotene			Cyt.	25.3	29.0	22.8	22.9	0.84	48.2
	Tetrads to	—		Nucl.	28.6	23.7	18.9	28.8	0.92	57.4
	Microspores	—		Cyt.	24.9	29.8	23.2	22.2	0.83	47.1

For *Trillium*, the microsporocytes from 150 anthers were suspended in 1.5 ml of incubation medium, for each assay, and 0.45 mc of P^{32}-phosphate added. The cells were incubated for 12 minutes at 4°C. For *Iris*, the microsporocytes were collected from 200 anthers and the microspores from 450 anthers; the isolated cells were respectively suspended in 2 and 4 ml of incubation medium containing 0.15 mc of P^{32}/ml and assayed at 15°C for a period of 12 minutes. Small Erlenmeyer vessels were used for all incubations. In the Actinomycin D experiment, the microsporocyte suspension was preincubated with the inhibitor (0.5 μg/ml) for 30 minutes at 4°C and then exposed to P^{32} for 12 minutes at 15°C. The values given are in moles/100 moles nucleotide. Other conditions are described in text.

ient one, because of the very small amounts of material available, was the nucleotide composition of RNA labeled with P^{32}-phosphate. On the basis of the preceding experiments, it was supposed that despite the slow rate of cell development a brief period of exposure would be necessary if messenger RNA were to be made apparent by the label. Since a large number of plants were necessary for a single experiment, no attempt was made to determine optimal exposure times. In these initial experiments a positive result would be sufficient justification for the exposure time arbitrarily chosen.

The experiments here reported (Table III) do not include some initial failures. It is unnecessary to account for the failures except to mention one important methodological point—the procedure for obtaining RNA fractions. The procedure will not be described in detail, simply because the details are still unclear. The main features may be simply described, however. Following incubation, the cells are sedimented, frozen to the temperature of solid carbon dioxide, and plunged into 10 to 20 times their volume of cold glycerol. The mixture is kept for about 14 to 18 hours at −5°C or lower, after which the cells are separated from the fluid and ground with 180-mesh carborundum in a small volume of glycerol:3 M sucrose (4:1 by volume) containing 0.005 M $CaCl_2$ and 0.01 M Tris buffer (pH 7.4). When most cells are broken, the thick slurry is diluted with glycerol:ethanol (70:30 by volume) and centrifuged at 11300 *g* for 20 minutes. The residue is resuspended in this glycerol:ethanol mixture and the operation repeated until the supernatant fluid contains little cellular material. The supernates are combined and diluted with approximately ⅓ their volume of ethanol, and centrifuged at high speed to yield a particle-free clear supernate. The resulting sediment is designated as "cytoplasmic fraction"; it contains few, if any, nuclei. The initial residue contains the nuclei plus assorted debris (widely assorted in homogenates of plant cells) and is designated as the "nuclear fraction." We wish to emphasize that this fraction most probably contains cytoplasmic components. Each of the residues is washed with 95 per cent ethanol. The cytoplasmic fraction is treated by the standard phenol procedure for preparing RNA (5). The nuclear fraction is first extracted with a solution containing 0.2 per cent sodium lauryl sulfate, 0.05 M Tris buffer (pH 7.4) and 0.005 M magnesium acetate. After removal of the insoluble material by centrifugation, the solution is treated with phenol in the same way as the cytoplasmic fraction. The RNA fractions thus prepared were dissolved in Tris-magnesium acetate buffer, shaken three times with ethyl ether to remove residual phenol, dialyzed overnight against buffer, and precipitated with two volumes of ethanol. The precipitates were hydrolyzed with KOH in the presence of carrier RNA and the ribonucleotides fractionated on a Dowex-1-formate column according to the procedure of Osawa *et al.* (6). The need for introducing carrier at this point in the procedure arises from the fact that microsporocytes pooled from 100 anthers contain approximately 0.28 mg RNA; the yield is less because of mechanical losses in glycerol fractionation. Carrier RNA is necessary not only for unambiguous identification of nucleotides in the eluates, but also for the succeeding step designed to remove traces of inorganic phosphate which may have persisted through earlier purification steps. 100 mg of Norit and 100 mg of "Hyflo" supercel were mixed in water and the suspension allowed to settle in a column, 3 to 4 mm diameter. The column was washed with approximately 40 ml of 0.05 M perchloric acid. Each of the nucleotide fractions was passed through the column (2 μmoles was set as the upper limit of capacity) and the column washed with 0.05 M perchloric acid. All eluates were checked for counts; if an appreciable number of counts were present, indicating contamination by inorganic phosphate, washing was continued until the eluate no longer had any count. Usually 7 ml of 3 per cent NH_4OH were sufficient to elute the nucleotide; occasionally a small faction appeared in the subsequent wash with 0.3 per cent NH_4OH.

Cell suspensions which were exposed for 12 minutes to P^{32} in the absence of Actinomycin D under conditions listed in Table III, showed the following distribution of label: 25,000 CPM in the RNA of the nuclear fraction and 17,000 CPM in the RNA of the cytoplasmic one. The result could be interpreted as indicating a primary labeling of nuclear RNA, but the uncertainty surrounding the cytological composition of "nuclear fraction" makes any such interpretation questionable. From the standpoint of nucleotide composition, however, the results are unambiguous. A comparison of the data in Tables III and IV will indicate at once that the RNA synthesized

in the nuclear fraction has a composition matching that of the DNA, whereas RNA synthesized in the cytoplasmic fraction is virtually identical with ribosomal RNA. Microsporocytes of *Iris* and *Trillium* behave similarly; *Iris* was used in these experiments because it was the only other source of microsporocytes available at the time. We have not yet checked the base composition of *Iris* DNA, but presumably it would fall within the grouping of other monocotyledonous plants analyzed in our laboratory, namely, about 60 per cent adenine-thymine.

Thus, although the cytological counterpart of the messenger-like RNA cannot be ascertained, the synthesis of such RNA during meiotic stages ranging from pachytene to diakinesis is patent. That similar results were obtained for tetrad-microspore stages is not at all surprising since it was previously shown (3) that this interval is marked by intense synthetic activities. The results are compatible with those on Actinomycin inhibition shown in Table II. Another set of experiments was, nevertheless, performed in order to test directly the effect of Actinomycin on the two categories of RNA. Three features of the results (Table III) are of interest: (1) The degree of inhibition (68 to 84 per cent) over a 12-minute labeling period is higher than that found earlier for a 1-hour period. This may be taken to indicate a more rapid rate of synthesis of the sensitive fraction. (2) Both DNA-like and ribosomal-like RNA are sensitive to Actinomycin. If the current interpretations of the molecular site of actinomycin inhibition are correct, then it must be assumed that both types of RNA are being transcribed from DNA. (3) The data, however, do not support the conclusion that all RNA in the cell is thus transcribed. The distinctive nucleotide composition of RNA synthesized in the presence of actinomycin may be attributed either to the existence of an alternative route or to an ineffectiveness of the inhibitor arising from other physiological factors. In general, the interpretations are based on a number of assumptions which, though not unwarranted, need much more examination.

TABLE IV

Base Composition of Nucleic Acids in Microsporocytes and Microspores of Monocotyledonous Plants

Plant	AT in DNA	Nucleotide composition of RNA				AU in RNA
		A	G	C	U	
	per cent					*per cent*
Trillium	61	—	—	—	—	
Lily	58.5	—	—	—	—	
Tulip	60.5	25.2	29.8	22.9	22.3	47.5

Methods for preparation and analysis of DNA will be published separately. The values listed for DNA are based upon density measurements in CsCl and hyperchromicity changes with temperature. RNA from tulip microspores was prepared by the phenol procedure (5) and analyzed after alkaline hydrolysis according to the methods of Osawa *et al.* (6). A manuscript containing a fuller report of DNA composition in higher plants is now in preparation; all plants thus far tested have a composition within the range of 58 to 62 per cent adenine-thymine. The limited data on RNA composition of microspores is thus far unavoidable. Large numbers are required for analysis of the unlabeled material; the data obtained from labeled material using carrier are contained in Table III, but these are open to the possibility that only selected fractions of the cellular RNA become labeled over the limited intervals during which exposure to isotope is feasible.

None of the data here obtained match the results to be expected if chloramphenicol and Actinomycin D were absolutely specific in their action and complete in their effectiveness. Since the "deviations" may be explained in a number of ways, we prefer to leave this particular subject to further experimentation.

DISCUSSION

The initial objective of this study—to determine the correctness of conclusions drawn from analyses of intact anthers—begs little comment. The correspondence in behavior of isolated microsporocytes and those maintained *in situ* leaves little room for doubt that RNA and proteins are syn-

thesized during meiosis. Moreover, whatever doubt might remain is removed by the pioneering studies of Taylor (11) already referred to in an earlier publication (3). In these autoradiographic analyses of meiosis in lily, the incorporation of C^{14}-glycine and C^{14}-orotic acid into the proteins and nucleic acids of microsporocytes during meiosis was clearly shown. Insofar as the data obtained by these two different techniques are comparable, the patterns observed may be considered to be identical. The relatively high activities during interphase reflect a common and characteristic situation in which the interval of nuclear division is marked by a comparative inertness of metabolic activity. To the extent that this situation is regarded as a description of the bold features of cell development, it is adequate for the purpose. It is inadequate, however, if more detailed questions are injected about the cellular cycle. Though we use the term "information" in the loosest possible way, it is patent that the information essential to meiosis is an evolutionary product and that a critical fraction of that information is taped in the genome. With respect to this, the question may be asked as to how and when the taped information is translated into actual performance. In the case of mitosis it is commonly presumed—and with some experimental justification—that the primary acts of translation are all accomplished by the time prophase is reached (10). In general, chromosome contraction and physiological dormancy of the genome are considered to be tightly bound characteristics. The results here obtained do not support this conclusion. Some genes, a highly limited number to be sure, are active midway in meiosis.

Two questions which follow from the inference about gene activity are as yet difficult to answer. There is first the need to ascertain whether the activities demonstrated are directly tied to the cycle itself. In a number of mitotic studies it has been found that interference with a cell by radiation or certain poisons at about the metaphase interval is usually reflected in a disturbance of the succeeding mitosis (12). The principal evidence we have on this question has already been published (3), and it merely consists of a demonstration that application of RNA or protein inhibitors to cultured anthers during meiosis can lead to a disturbance of the cycle. The same set of observations furnishes a sole guide to answering the second question: What specific events are initiated by the genes during meiosis? From published observations and from more detailed ones currently being made (C. L. Kemp), it would appear that it is impossible to interfere with pairing and associated events up to metaphase I by administering a variety of inhibitors after leptotene has been initiated. It may be supposed, therefore, that the syntheses which are observed during pachytene and diplotene are related to one or more of the events beginning with the separation of chromosomes at metaphase I. This is of little value as a guide to meiotic mechanisms, but without a specific identification of the proteins formed there is no other.

Finally, there is the interesting question about the rates of molecular reactions and their bearing on the rates of comparable cytological events in different organisms. A simple parallel cannot be drawn between the generation time of a cell and the rates of underlying molecular processes. Since individual molecular reactions respond in more or less similar fashion to temperature change, it is reasonable to presume, on the basis of the results here obtained, that the spacing of the component events of meiosis is governed by a distinctive set of regulatory mechanisms. The slow motion of meiosis in *Trillium* is only partly attributable to the low temperature at which it occurs; the spacing of associated molecular events is much closer than might be supposed from the extended duration of the meiotic cycle.

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