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Ribonucleic Acid-Polyphosphate From Algae I. Isolation & Physiology [1, 2]

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An RNA-polyphosphate complex has been isolated from Mycobacteria by Winder and Denneny (31) and from Aspergillus by Belosersky and Kulaev (3). Both groups of investigators postulated that chemical bonds existed between the RNA and the polyphosphate. P^{32}-phosphate was rapidly incorporated into acid-insoluble RNA-polyphosphates in Aspergillus (22) and the specific activity of the polyphosphate was shown to be higher than the specific activity of the RNA. In the case of Azotobacter, Zaetseva, and Belozersky (32) showed a similar pattern of incorporation of P^{32} into the acid-insoluble polyphosphates and the only phosphate fraction labeled more rapidly was ATP. These properties indicate that a metabolic importance should be associated with the RNA-polyphosphate fraction from microorganisms.

No careful purification or detailed characterization of an RNA-polyphosphate has been reported. Also no convincing evidence for a metabolic role for these complexes has been demonstrated. However, all reports indicate that the RNA-polyphosphates are major components of the phosphorus and nucleic acid fractions from microorganisms. These reports also agree that there is a rapid turnover of the polyphosphate in these complexes. Consequently, this paper presents results of a more detailed study of the isolation and properties of RNA-polyphosphate with some speculations about its possible metabolic role.

Many reports have established that polyphosphates are normal constituents of algae (1, 9, 13, 18, 25, 27, 28, 29). In Acetabularia the polyphosphates have been found in spheres in the cytoplasm (29), while in Zygnemataceae the polyphosphates were found in the chloroplasts along with a high concentration of RNA (18). Polyphosphates have been reported in nuclear equivalents which contain RNA and DNA, in addition, and which have also been referred to as pseudovacuoles (13, 21). In all these previous reports fractions containing only polyphosphates and those containing both the polyphosphate and RNA have not been differentiated.

Materials & Methods

► Anabaena Culture. An inoculum of the blue-green alga, *Anabaena variabilis* Kütz, was obtained from the Algal Culture Laboratory, Botany Department, University of Indiana. This alga was mass cultured in 6-liter flasks with medium C, as described by Kratz and Myers (20). The cultures at 30 C

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were aerated with 1 % CO_2 in air, and illuminated with 500 ft-c of continuous light from incandescent bulbs. Details of the mechanics of the culture system are reported elsewhere (7). For harvest, the culture medium was centrifuged in a refrigerated centrifuge, the cells resuspended in cold distilled water and centrifuged for 6 minutes at 1,770 × *g* to establish their packed-cell volume, p.c.v.

The correlation between age of culture, p.c.v., and percentage cells in a state of division is shown in figure 1. After about ten days conditions became growth limiting, resulting in a high proportion of cells in a state of division. The increase in packed cell volume in cultures over 9 or 10 days of age was largely caused by the production of an excessive amount of sheath-material encasing the filaments.

► Chlorella Culture. A culture of *Chlorella pyrenoidosa* V.N. 2.2.1 and suggested conditions for synchronous growth were obtained from R. R. Schmidt, Department of Biochemistry and Nutrition, Virginia Polytechnic Institute. The apparatus for mass synchronization of Chlorella was designed and constructed from plexiglass so that 20 liters of culture could be maintained at 25 C under light saturations conditions from daylight-type fluorescent bulbs and aerated with 5 % CO_2 in air (7). Chlorella synchronization was induced by raising a 600 ml subculture in a separate apparatus to a density of about one milliliter p.c.v. per 100 ml of medium which was sufficient to keep at least 80 % of the cells at the young daughter-cell stage. These cells were removed by centrifugation and resuspended in 20 liters of fresh medium in the synchronization apparatus. The dilute algal suspension was first subjected to 800 ft-c of light for 18 hours and then to 12 hours of darkness. Thereafter, the culture was maintained on a 30-hour cycle consisting of 18 hours of 1500 ft-c of light followed by 12 hours of darkness. After four light cycles the cells were considered adequately synchronized and harvests were begun. Usually 15 or 16 liters of culture were harvested and the remainder was diluted to 20 liters with fresh medium for continuation of the culture in its synchronous state. Harvesting of the Chlorella was carried out with a Sharples continuous centrifuge with a large jet. The packed algae were transferred to 100 ml tubes and centrifuged in a refrigerated centrifuge, washed once with distilled water, and finally centrifuged at 2,120 × *g* for 10 minutes to establish their p.c.v. They were stored frozen until used for analyses.

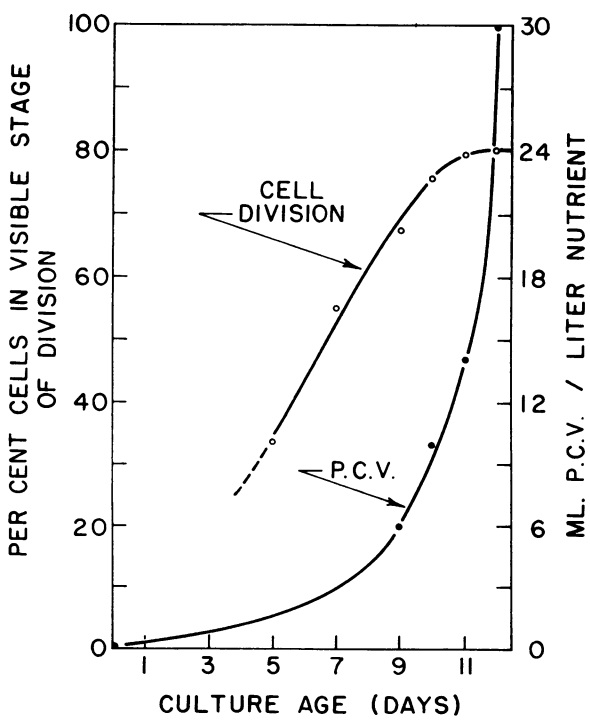

Fig. 1. Increases in packed cell volume and percentage dividing cells in a typical Anabaena population during growth.

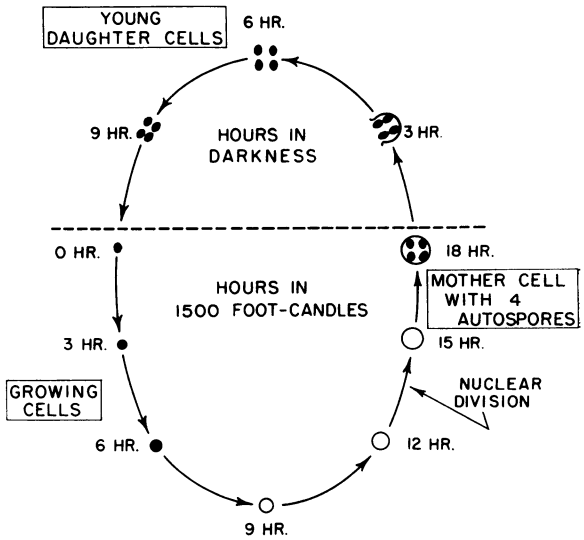

Fig. 2. Synchronization cycle of *Chlorella pyrenoidosa* (Van Niel 2.2.1) at 25 C.

Chlorella cells were harvested and RNA-polyphosphate isolations were performed for every 3 hours in the life cycle of 30 hours. The type of Chlorella cells which predominated at each of the harvest periods during the synchronization cycle is shown in figure 2. These harvests could not be made consecutively during one life cycle of the culture, for it was necessary to obtain about 15 to 30 ml of p.c.v. each time for analyses. Therefore, each harvest at the selected stage of growth was made after at least three to four complete cycles of growth. In one case a culture was maintained for 21 hours in light before harvesting. In another case 40 ppm of chloramphenical was added to the culture after 9 hours of light and the algae were harvested at the 12-hour light stage.

► Fractionation Procedures for Phosphorus Components. In figure 3 the methods used to fractionate the phosphorus components of Anabaena are outlined. This procedure, up to the crude preparation for the polyphosphate-RNA, was a slight modification of the procedures reported by Kulaev and Belozersky (22) and Juni et al. (17). The phenol extraction has been described by Gierer and Schramm (11) and the final material has been designated as the phenol preparation.

The procedure developed for isolating the RNA-polyphosphate from Chlorella was also based upon

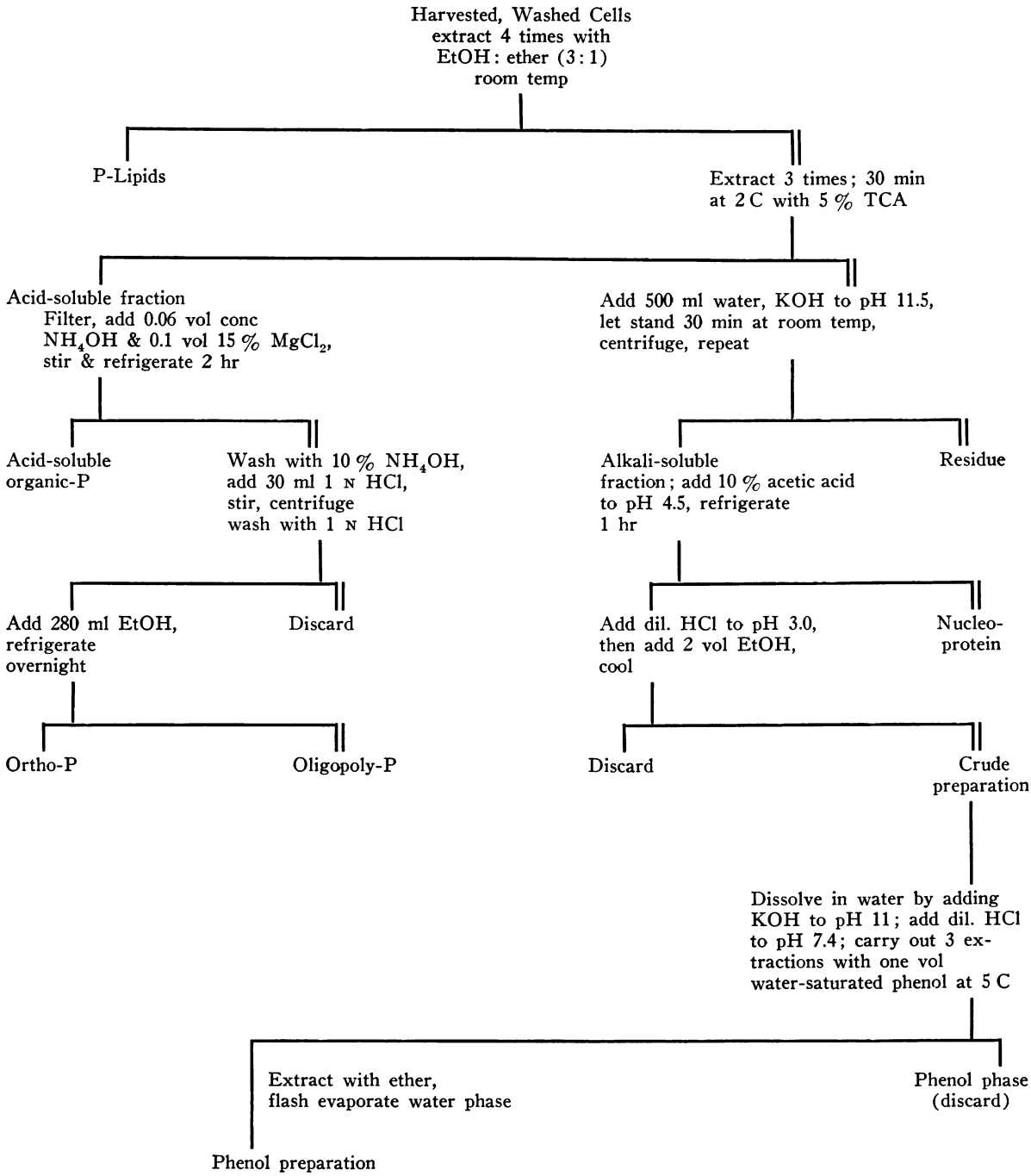

Fig. 3. Fractionation of the phosphorus components of Anabaena.

phenol extractions. A frozen sample of algae was thawed in 300 ml of distilled water which was adjusted (at intervals) to pH 11.5 with KOH. This initial treatment solublized but did not hydrolyze the polyphosphate-RNA material. After 1 hour at room temperature the pH was adjusted to 7.4 with HCl and 300 ml of cold, water-saturated phenol was added. The mixture was shaken for 5 minutes and then centrifuged for 8 minutes at 5 C. The upper (aqueous) phase was saved, 300 ml of cold water-saturated phenol was added, and the extraction repeated. The combined aqueous phase was then extracted four times with ether and evaporated under reduced pressure for a short time to remove the residual ether. Enough sodium acetate was added to make a 1 % solution, and one drop of 2 N HCl and four volumes of alcohol were then added. Additional sodium acetate was added slowly with stirring until a flocculent precipitate formed which contained the RNA-polyphosphates. The solution was then put in a freezer for 1 hour with occasional stirring. The precipitate was removed by centrifugation at 0 C, washed with alcohol, and dried at reduced pressure.

► DEAE-Cellulose Chromatography. The polyphosphate-RNA obtained from the solvent fractionation procedures was further separated on a diethylaminoethylcellulose (DEAE-cellulose) column. Prior to use the DEAE-cellulose was washed in a large column first with a saturated NaCl solution, then with 0.1 N NaOH, and finally with a large volume of 0.01 M tris-hydroxymethyl-amino-methane (tris) buffer, pH 7.6, until the pH of the eluate was 7.6. Columns 20 cm long and either 2 cm in diameter for material from Anabaena or 1 cm in diameter for material from Chlorella were packed with only very gentle pressure and washed with a small volume of the tris buffer. The precipitate from a fractionation procedure with Chlorella was dissolved in 30 ml of the tris buffer, applied to the column, and washed with 20 ml of the buffer. The columns were connected to a 500 ml mixing flask which was filled with the tris buffer and the mixing flask was in turn connected to a reservoir of a salt solution. For separation of material from Anabaena, 2 M NaCl was added to the reservoir, and 10 ml fractions were collected from the column. In the work with Chlorella, a series of NaCl solutions was added in sequence to the reservoir: 200 ml of 1 M NaCl, 200 ml of 2 M NaCl, 200 ml of 3 M NaCl, and 200 ml of 4 M NaCl. Fractions were collected (5 ml) from the columns.

► Analyses. Total phosphorus was determined by the technique of King (19). Acid-labile phosphate-phosphorus was determined by hydrolyzing samples for 7 minutes with 1 N HCl in a boiling water bath and then determining orthophosphate by the method of Fiske and Subbarow (10). For the RNA-polyphosphate fractions the nonacid-labile phosphorus was obtained by the difference between these two analyses (difference-P).

Ribose was determined by the orcinol reaction (6) using a 40-minute hydrolysis with 6 N HCl in a boiling water bath. A solution of adenylic acid was used as a standard. Deoxyribose was tested for by the diphenylamine reaction (5). Total nucleic acid was determined by the method of Webb (30). Protein was determined by both the method of Lowry et al. (23) using bovine serum albumin as a standard, and by the ninhydrin reaction.

Optical densities in the ultraviolet region were measured with a Beckman, model DU, spectrophotometer with 1 cm quartz cells. An OD unit of RNA was equivalent to the amount of material in 1 ml which had an absorbance of 1.0 at 260 mμ. The RNA in the fractions was hydrolzed to the free nucleotides by incubation for 18 hours with 0.5 N KOH at 40 C. Subsequent neutralization with cold 36 % $HClO_4$ resulted in the precipitation of $KClO_4$ which was filtered off. The filtrate was subjected to column chromatography by the method of Hurlbert et al. (14). The separated nucleotide solutions were then lyophilized to remove the formic acid. Ultraviolet spectra were determined at both acidic and basic pH to establish identities. The major nucleotides were also identified by subjecting the RNA in the fractions to 1 hour of hydrolysis with 1 N HCl in sealed tubes in a boiling water bath. The hydrolysates were then chromatographed on Whatman No. 1 paper by the descending technique with an isobutyric acid-NH_4OH-water (66/1/33) solvent (26).

Results

► RNA-Polyphosphate From Anabaena. Distribution of phosphorus in the fractions obtained from the isolation procedure (fig 3) are shown in table I for two cultures of Anabaena of different ages as represented by their p.c.v. In both cases the combined

Table I

Distribution of Phosphorus in Anabaena Harvested at 2 Culture Densities

Phosphorus fractions	Culture No. 1 (density = 5.6 ml p.c.v.*/l)		Culture No. 2 (density = 12 ml p.c.v./l)	
	μg P/ml p.c.v.	%	μg P/ml p.c.v.	%
P-lipids	2	0.3	8	1.4
Acid soluble organic-P	68	10.2	5	0.9
Residue	29	4.3	77	13.1
Ortho-P	224	33.5	175	29.9
Oligopoly-P	130	19.5	153	26.1
RNA-poly-P (crude prep.)	215	32.5	168	28.7
	(134)**	(20.2)**		
Total P	668	100	586	100

* p.c.v. = packed cell volume.
** Figures in parenthesis are $\triangle_7$-P values and represent the labile polyphosphate portion of this fraction.

Table II

Parameters of Typical Phenol Preparations of RNA-Polyphosphate From Anabaena

Parameter	Experiment							
	1	2	3	4	5	6	7	8
A. Total-P (μg P/ml p.c.v.)	95	64	83	41	216	116	451	77
B. $\triangle_7$-P (μg P/ml p.c.v)	81	40	71	33	134	61	366	52
C. Difference-P (μg P/ml p.c.v.)	14	24	12	8	82	55	85	25
D. ($\triangle_7$-P)/(diff.-P)	5.8	1.6	6.0	3.9	1.7	1.1	4.3	2.1
E. Total nucleic acid (μg/ml p.c.v.)	...	...	210	56	1,060	272	1,380	228
F. Calculated nucleic acid P: (nucleic acid) $\times$ (1/10.3)	...	...	20	8	103	26	134	22
G. Ribose (orcinol test) (μg/ml p.c.v.)	105	...	69	42	...	...	...	...
H. Calculated ribose: (diff.-P) (150/31)	67	...	60	40	...	...	...	...

oligo- and RNA-polyphosphates constituted about 40 to 50 % of the total phosphorus and the bulk of the remaining phosphorus was orthophosphate.

Phenol preparations of the RNA-polyphosphate component from a number of Anabaena cultures were analyzed (table II). Protein was either absent or only present in trace amounts. The total phosphorus (parameter A) and labile phosphorus (B) varied among the Anabaena cultures, but there was always more labile phosphorus than stable phosphorus in these fractions as illustrated by the ratio of 7-minute acid-labile phosphorus to difference phosphorus (D). The amount of stable RNA-phosphorus was estimated by the difference between the total and acid-labile phosphorus (C) or by multiplying the amount of nucleic acid (E) by the gravimetric factor, 1/10.3. The amount of nucleic acid phosphorus was of the same magnitude by either method of calculation (C & F). This approximate correspondence indicated that major amounts of other phosphorus compounds were not present as contaminants. Also a correspondence was found between ribose as determined with the orcinol reaction (G) and ribose as calculated (H) by multiplying the stable phosphorus by the appropriate gravimetric factor. For different experiments, considerable variability among the parameters examined reflected large biological variations in the algae. Subsequent data with Chlorella has indicated that these differences were probably caused by variation in the stage of growth of the Anabaena at the moment of harvest.

No significant contamination by DNA existed, since no deoxyribose was found in crude preparations of RNA-polyphosphate from Anabaena. When an aliquot of a phenol preparation of RNA-polyphosphate was subjected to alkaline hydrolysis and the nucleotides were resolved by ion-exchange chromatography, the following mole percentages were obtained: CMP, 22.8; AMP, 20.9; GMP, 31.6, and UMP, 24.7. Acid hydrolysates of Anabaena RNA-polyphosphate were also prepared and separated by paper chromatography. Adenine, guanine, cytidylic acid, and uridylic acid were identified, but no thymidylic acid was detected.

► DEAE-Cellulose Chromatography of RNA-polyphosphate From Anabaena. The RNA-polyphosphate fractions from seven Anabaena cultures of increasing density or p.c.v. were isolated as phenol preparations (fig 3). These preparations were further fractionated on DEAE-cellulose columns and the elution patterns as measured by the optical densities at 260 mμ and total phosphorus are shown for three representative cultures in figure 4. The amount of polyphosphate from the young culture (4

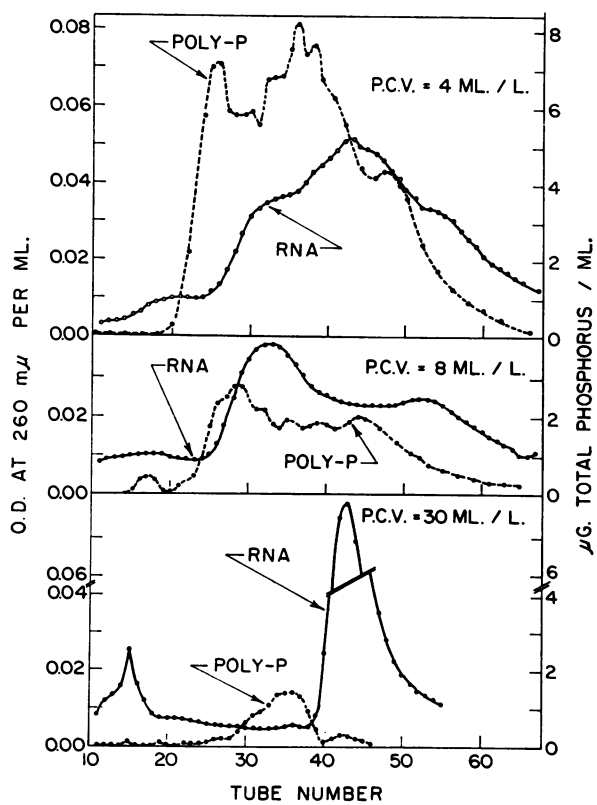

Fig. 4. Elution patterns of RNA-polyphosphate obtained from Anabaena cultures of various densities upon DEAE-cellulose chromatography.

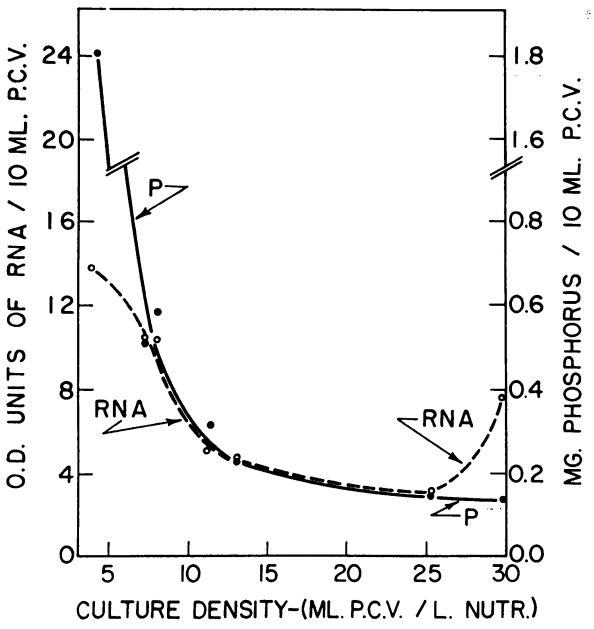

Fig. 5. Total acid-insoluble RNA and phosphorus per 10 ml p.c.v. in Anabaena cultures of different densities. One RNA OD unit is equivalent to 1 ml of solution with an absorbance of 1.0 at 260 mμ.

ml p.c.v.) was much greater than from the old culture (30 ml p.c.v.). From the old culture there was a large RNA peak, which apparently contained very little polyphosphate.

The total phosphorus and total OD units of RNA for all the DEAE-cellulose fractionations from each Anabaena culture were plotted against culture density, representing culture age, on a scale selected for comparative purposes (fig 5). This relationship of the total RNA to total phosphorus, which was predominantly acid-labile polyphosphate, could be divided into three parts. In the younger cultures (about 5 ml p.c.v. per liter nutrient) there was an excessive amount of polyphosphate, even though there was also a relatively large amount of RNA. A second condition was represented by rapidly growing cultures in which the ratio of OD units of RNA to mg of total phosphate was fairly constant. Finally in the very old cultures of about 30 ml p.c.v. per liter the amount of RNA increased without a corresponding increase in polyphosphate.

Large amounts of amino acids and peptides have been found covalently bonded to RNA as usually isolated (12, 15). However, the Anabaena RNA fractions separated in this study on DEAE-cellulose columns gave no detectable ninhydrin reaction before or after a 1-hour hydrolysis in 2 N HCl at 100 C.

► RNA-Polyphosphate Complexes From Synchronous Chlorella. Because of wide variability in the RNA-polyphosphate content from Anabaena of different ages, a synchronous culture of Chlorella was used as a source of material. Most of the Chlorella cells at one harvest time were of the same age, but each harvest time at 3-hour intervals represented a different stage in the life cycle of this alga (fig 2). The RNA-polyphosphate fractions were initially extracted by the phenol procedure described in the methods section. Afterwards, they were chromatographed on DEAE-cellulose columns and the sums of the RNA and phosphorus from each chromatographic fractionation are shown in figure 6. Al-

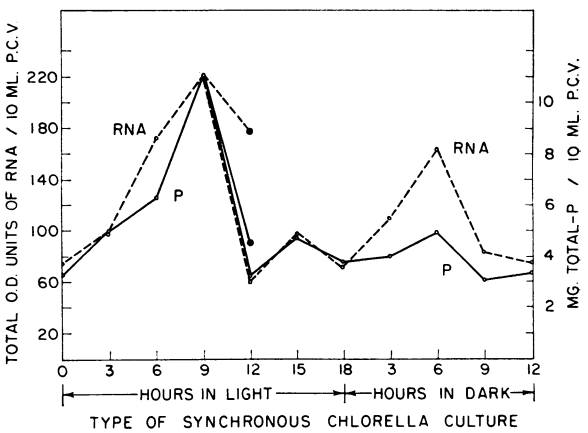

Fig. 6. Total RNA and phosphorus per 10 ml p.c.v. of Chlorella during synchronous growth. In one case (●) chloramphenicol was added at nine hours of light. One RNA OD unit is equivalent to 1 ml of solution with an absorbance of 1.0 at 260 mμ.

though the data in figure 6 are on the basis of p.c.v., during one life cycle the p.c.v. increased two- or three-fold (16), so that total RNA or total phosphorus per culture would have shown even greater increases during the first 9 hours of growth. There was a close correlation between changes in amount of total phosphorus and amount of total RNA in these fractions on the basis of the algal p.c.v. at each stage in the Chlorella life cycle. A three-fold increase in the amount of RNA and polyphosphate per milliliter p.c.v. occurred during the first 9 hours in the light, when the cells grew in size but not in number. Between 9 and 12 hours in the light, a large decrease in the RNA and polyphosphate took place, when the cells should have been preparing for nuclear division.

DEAE-cellulose elution patterns from eight representative stages in the Chlorella life cycle were determined by optical densities at 260 mμ and total phosphorus (fig 7 & 8). For each fractionation, six elution areas of RNA and polyphosphate, labeled A and F on the figures, were selected for analysis, since they were eluted at about the same place in each experiment and since the amounts of each showed systematic rather than erratic changes. Changes in RNA in these six areas (A–F) during the life cycle

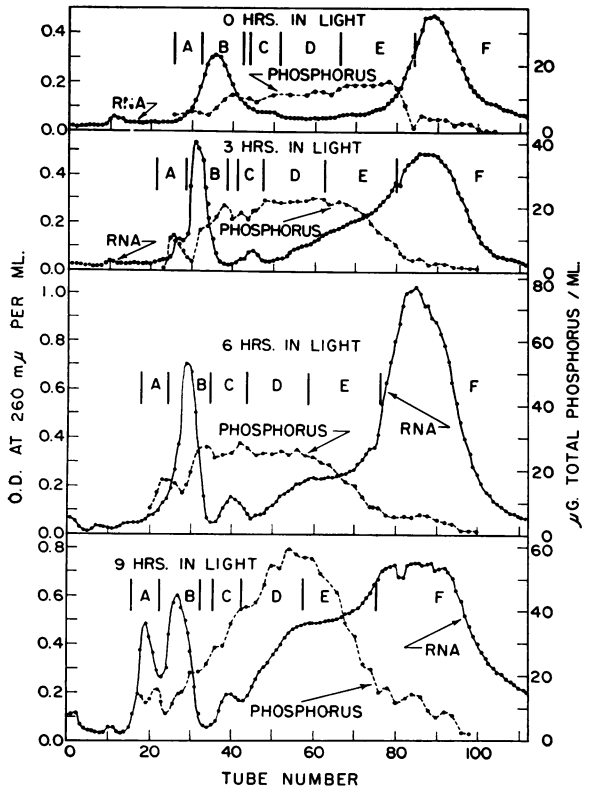

Fig. 7. Elution patterns of RNA-polyphosphate per 10 ml p.c.v. of Chlorella from synchronous cultures of designate state in their life cycle (fig 2). DEAE-Cellulose fractions A to F are designated above each elution pattern.

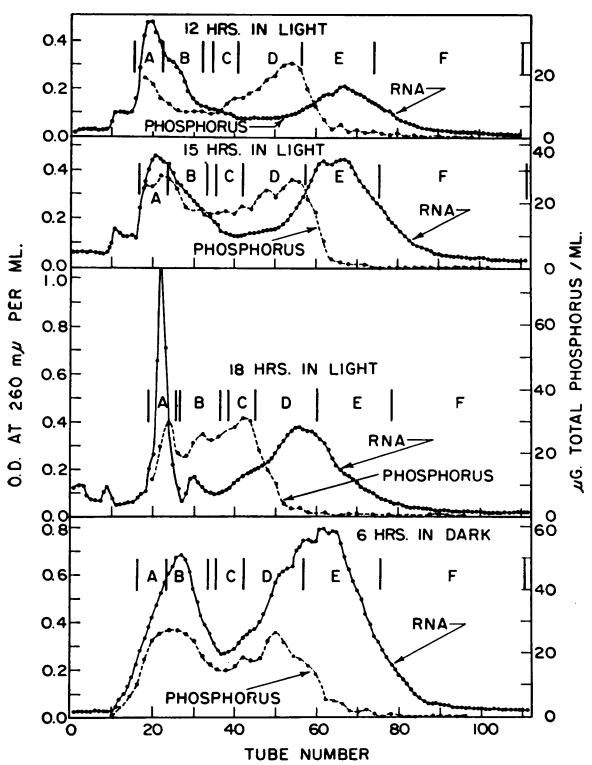

Fig. 8. Elution patterns of RNA-polyphosphate per 10 ml p.c.v. of Chlorella from synchronous cultures. DEAE-cellulose fractions A to F are designated above each elution pattern.

of Chlorella are illustrated in figure 9. All the RNA components increased in total amount during 0 to 9 hours of light and areas D, E, and F decreased sharply between 9 and 12 hours of light. Areas B, D, and E again increased during the first 6 hours in the dark. Area F, which was probably of highest molecular weight, because it required the highest salt concentration for elution, showed the greatest changes. Treatment with chloramphenicol from the 9- to the 12-hour light period prevented a rapid decrease in the RNA content of areas D and E which normally occurred during this period. In fact, the RNA in areas D and E continued to increase during this 3-hour treatment at a rate about equivalent to the previous 9-hour cell growth phase. However, the chloramphenicol had little influence upon the rapid disappearance of the large RNA fraction in area F after

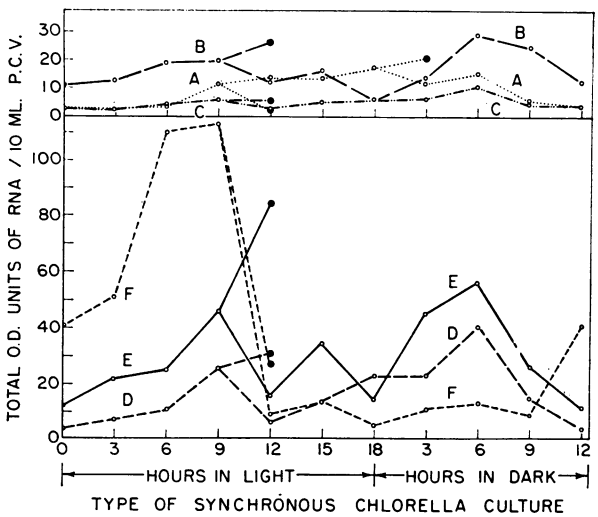

Fig. 9. Changes in Chlorella RNA during synchronous growth. In one case (●) chloramphenicol was added at nine hours of light for 3 hours before harvest. DEAE-Cellulose fractions labeled A through F were from figures 7 and 8.

the 9-hour light stage. In the dark part of the life cycle there was a build-up of RNA in the first 6 hours, and in the last 6 hours of darkness, the RNA content returned to a low level. In order to maintain a viable culture a dark period or one of low light intensity was necessary. During this dark period changes did occur in total RNA and in qualitative distribution among the different chromatographic fractions.

Changes in the amount of total phosphorus in each of the six elution areas from the DEAE-cellulose columns are shown in figure 10. As with RNA, phosphorus, mainly as polyphosphate, increased in all areas during the first 9 hours in the light and then all but area A decreased rapidly between 9 and 12 hours in the light. The amounts of polyphosphate in areas D and E were by far the largest, whereas the largest RNA fraction was F. There was, however, a significant amount of polyphosphate in all areas except F which contained little phosphorus after 12 hours of light. Chloramphenicol treatment at the

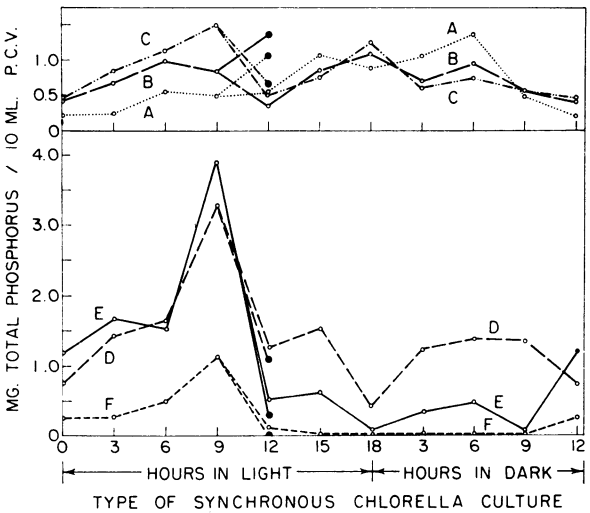

Fig. 10. Changes in total phosphorus of Chlorella RNA-polyphosphate fractions. DEAE-Cellulose fractions are labeled A to F.

9-hour light period did not influence the loss of polyphosphates from areas D, E, and F although it did prevent RNA loss from areas D and E.

► Ratios of RNA to Polyphosphates. In table III the amounts of total RNA, total phosphorus, and acid-labile phosphorus are summarized from many Anabaena and Chlorella phenol preparations which had been fractionated on DEAE-cellulose columns. The values are the sum of the combined areas of the total elution pattern. The ratio of 20 RNA optical density units per milligram of total phosphorus in the total RNA-polyphosphate present in the middle part of the Anabaena growth curve (fig 5) was about the same as in synchronous Chlorella in the light growth phase of their life cycle. This similarity seems significant, although it may be a coincidence of our limited data. The low ratio observed in Chlorella kept in continuous high light was caused by a decrease in total RNA rather than an increase in total phosphorus.

Each combined eluate area from a DEAE-cellulose column fractionation of the RNA-polyphosphates from a Chlorella culture was analyzed for ribose and deoxyribose. No deoxyribose could be found. Ribose was found in each eluted fraction. From areas D and E the μg ribose per RNA OD unit calculated as 13 from area D and 17 μg from area E, and a value of 15 μg or 0.1 μmole ribose per RNA OD unit was arbitrarily taken for approximate calculations. Since only purine nucleotides were determined in the orcinol reaction, this value should be approximately doubled (0.2 μmoles total ribose per RNA OD unit). These calculations were carried out in order to attach a physical meaning to the ratio of 20 RNA OD units per mg total phosphorus (table III). Therefore, one RNA OD unit was equivalent to 50 μg or 1.6 μmole of phosphorus. Thus for every micromole of ribose in the total system of complexes there were about eight micromoles of phosphorus of which 7 micromoles would be polyphosphate-phosphorus. This calculated ratio of RNA phosphorus to polyphosphate-phosphorus may be in considerable error, but

Table III

Influence of Light Upon Ratio of Total RNA OD Units to mg Total Phosphorus*

Culture conditions	RNA units/ 10 ml p.c.v.	mg total-P/ 10 ml p.c.v.	mg$\triangle_7$-P/ 10 ml p.c.v.	$\triangle_7$-P Diff.-P	RNA units/ mg total-P
		Anabaena			
3.6 ml p.c.v./l	14	1.8	...	...	8
7.2 ml p.c.v./l	10	0.5	...	...	20
8.0 ml p.c.v./l	10	0.6	...	...	18
11.2 ml p.c.v./l	5	0.3	...	...	16
13.0 ml p.c.v./l	5	0.2	...	...	20
25.0 ml p.c.v./l	3	0.1	...	...	21
29.6 ml p.c.v./l	8	0.1	...	...	58
		Chlorella			
Synchronized, 0 hr light	75	3.3	2.6	3.4	23
Synchronized, 3 hr light	98	5.1	4.7	11.5	19
Synchronized, 6 hr light	172	6.3	5.9	15.6	27
Synchronized, 9 hr light	220	11.1	9.4	5.7	20
Synchronized, 12 hr light	60	3.2	3.0	15.2	18
Synchronized, 15 hr light	97	4.8	4.3	8.1	20
Synchronized, 18 hr light	71	3.7	3.3	8.1	19
Synchronized, 21 hr light	99	5.3	4.7	7.3	19
Synchronized, 3 hr dark	109	4.0	3.2	4.6	28
Synchronized, 6 hr dark	163	4.9	2.7	1.2	33
Synchronized, 9 hr dark	82	3.1	2.6	5.9	27
Synchronized, 9 hr light plus chloramphenicol	177	4.5	3.8	5.7	40
Random-high continuous light	38	4.0	3.5	7.8	10
Random-low continuous light	268	7.9	5.9	3.0	34

* One RNA OD unit = 1 ml of a solution with an OD, 260 mμ, of 1.0.

it serves to emphasize that there was much more polyphosphate than RNA-phosphorus present.

The ratio of 7-minute acid-labile phosphorus to difference-phosphorus (tables II & III) varied from about 1 to 16 and was usually 8 or less. Acid hydrolysis should have resulted in the release of all phosphate which was bound only by anhydride linkages.

Discussion

Material isolated from Anabaena and Chlorella by solvent extractions and then DEAE-cellulose ion exchange column chromatography contained both RNA and polyphosphate. This fraction contained between 25 to 35 % of the total phosphorus in Anabaena and a major portion of the alga's RNA. No DNA, amino acids, protein or significant amounts of other phosphorus compounds were detectable. RNA was identified by alkaline hydrolysis to the free nucleotides which were isolated by column chromatography and identified by their ultraviolet spectra. Adenine, guanine, cytidylic acid, and uridylic acid were also identified by paper chromatography of acid hydrolysates. Polyphosphate was identified by such classical characteristics as alkali-stability, acid-lability, precipitation with barium ion at pH 4.0, and strong metachromatic reaction with toluidine blue. Thus, RNA and polyphosphate were the only two components identified in the isolated material.

When RNA-polyphosphate isolated from Chlorella was chromatographed on DEAE-cellulose columns, the material separated into numerous fractions. Six elution areas were selected because of consistency in chromatographic position and systematic changes during synchronous growth of the algae. Each area contained both the RNA and polyphosphate components. However, the relative amount of material in each area depended upon the stage of algae growth. Further, the relative distribution between RNA and polyphosphate was not the same in the different fractions. Area F, which eluted at the highest salt concentration, contained a small amount of polyphosphate relative to its RNA content. Areas D and E, in contrast, always contained a large amount of polyphosphate. During the first 9 hours of light both RNA and polyphosphate synthesis were rapid. During the first 6 hours the amount of RNA in area F increased most rapidly (fig 9), but during the period of 6 to 9 hours RNA in area F did not increase significantly, while RNA components in D and E continued to increase. These data suggest that the RNA portion of the fractions was synthesized first and was isolated in area F. The polyphosphate for area D and E may then have been elaborated by an enzymatic process involving ATP with a consequential modification of the chromatographic properties of the RNA. The resulting RNA-polyphosphate was then found in areas D and E. This hypothesis is supported by polyphosphate synthesis which was more rapid from 6 to 9 hours of light than between 0 and 6 hours. This hypothesis is consistent with speculations by other investigators (2, 4, 8), that polyphosphates are synthesized on the surface of RNA.

A large synthesis of Chlorella RNA was noted during the first 6 hours of darkness without a corresponding synthesis of polyphosphate. One wonders about the function of this RNA, since it was synthesized and lost during a period of dark starvation and suspended growth of the cells. A similar situation occurred in a large synthesis of RNA by an auxophilic mutant of *Escherichia coli* when methionine was withheld (24). When methionine was added to that deficient medium, no protein synthesis occurred for some time suggesting that the accumulated RNA was nonfunctional.

A ratio of about 1 mole of nucleotides to 7 moles of polyphosphate-phosphorus was consistently found in the total RNA-polyphosphate fractions from both Chlorella and Anabaena providing the algae cultures were in a state of rapid growth and not under the influence of abnormal environments. These calculations were based upon total RNA analysis by OD and ribose as compared to total phosphorus. Variations from the ratio of 7 polyphosphate-phosphorus per nucleotide were noted for Chlorella at three stages of growth. In early light stages polyphosphate synthesis lagged behind RNA synthesis as was reflected by a reduction of the ratio to 5. This ratio dropped to 3 polyphosphate-phosphorus per nucleotide when the algae were treated with chloramphenicol because of a failure of normal RNA utilization. In the dark the algae accumulated RNA without a build-up of polyphosphate and the ratio was about 4 after 6 hours of darkness.

The complex changes in the RNA-polyphosphate fractions cannot now be precisely correlated with stages of cellular growth and division. However, the increase in RNA-polyphosphate in the first 9 hours of light could simply involve a preparation for certain energy requiring syntheses which take place between 9 and 12 hours of light when the RNA-polyphosphate was rapidly utilized. This utilization just preceded nuclear division at 14 hours of light. The correlation between RNA-polyphosphate content and the stages of Chlorella growth seems very significant, but biological variations must be considered since each 3-hour harvest was taken 3 or 4 days apart during several weeks in order to obtain sufficient material for the analyses.

Summary

Algae, Anabaena, and Chlorella, were mass cultured for the isolation of RNA-polyphosphate fractions. In actively growing Anabaena cells total polyphosphates constituted about 40 to 50 % of the total phosphorus, and the polyphosphate in the RNA-polyphosphate fraction amounted to about 30 %. The isolation consisted of solvent extraction procedures followed by DEAE-cellulose chromatographic fractionation into a spectrum of RNA-polyphosphate types. Six areas from the column chromatograms were then analyzed for amounts of RNA and polyphosphate. No other components were detected.

All of the chromatographic areas contained both RNA and polyphosphate. Within the total RNA-polphosphate complex there was about one nucleotide to seven polyphosphate phosphorus from both Chlorella and Anabaena providing the algae cultures were in a state of rapid growth.

The relative amount of RNA-polyphosphate in each chromatographic area depended upon the stage of synchronous Chlorella growth. During the first 9 hours of light, RNA-polyphosphate synthesis was rapid and accumulated in the three areas that eluted with the highest salt concentration. During the next 3 hours these fractions decreased while the cells were preparing for nuclear division. Chloramphenicol prevented the decrease in RNA at that time without affecting polyphosphate utilization. Significant changes also occurred during the rest of the Chlorella life cycle.

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