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TRANSCRIPTION DURING SPHERE-ROD MORPHOGENESIS OF ARTHROBACTER

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TRANSCRIPTION DURING SPHERE-ROD

MORPHOGENESIS OF ARTHROBACTER

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To the memory of Dr. Glenn Carmer Couch, who through the University of Oklahoma Scholars Program and his personal example challenged me to find my greatest potential in academic endeavors, which has led to the completion of this work.

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TRANSCRIPTION DURING SPHERE-ROD MORPHOGENESIS OF *ARTHROBACTER*

CHAPTER I

INTRODUCTION

The genus *Arthrobacter* is distinguished from other genera of the Corynebacteriaceae by the sphere-rod morphogenesis it undergoes under certain conditions (10,29). When stationary phase spherical cells are transferred to fresh medium, the cells germinate into rods. After a period of growth as rods, the cells synthesize multiple cross walls and divide into bacillary cells, which gradually shorten into spheres. Ensign and Wolfe (14) found that in glucose-minimal salts medium *Arthobacter crystallopoietes* grew and divided as spheres, but the addition of any of a number of apparently unrelated compounds induced elongation and growth as rods. When the inducer was depleted the rods gradually shortened into spheres.

Several biochemical events are known to be associated with morphogenesis. Ferdinandus (15) found that *Arthobacter crystallopoietes* accumulated fats during rod-formation and utilized them as an endogenous energy source during

rod-formation and utilized them as an endogenous energy source during rod-shortening. Lipolytic and gluconeogenic enzymes had highest specific activities during rod-fragmentation. The lipogenic enzymes, malic enzyme and glucose-6-phosphate dehydrogenase, reached highest specific activities during rod-formation. Malic enzyme was completely absent during coccoidal growth. Krulwich and Ensign (23) found that glucose permease was inducible by glucose but repressed in cells grown in glucose-minimal-salts plus succinate, an inducer of rod-formation. Krulwich and Ensign (22) also found the specific activity of an autolytic N-acetyl muramidase in the cell walls decreased 4 to 5 times during rod-formation. Krulwich, et al. (20) reported that the polysaccharide backbones of the rod peptidoglycans were longer and more homogeneous in length than those of the spheres. An additional glycine was present in the peptide cross-bridges of the sphere (21). Recently, Dalbow (12) found specific activity of tRNA methylases decreased during growth as rods.

Although many biochemical changes can be associated with the morphological changes in Arthrobacter crystallopoietes little is known of control mechanisms functioning during morphogenesis. Changes in specific activity of enzymes may be due to either feedback inhibition or repression. Ferdinandus and Clark (16) reported that free fatty acids were at the highest concentration during rod-shortening, and that free fatty acids inhibited lipogenic

enzymes *in vitro*. No studies on transcription during sphere-rod morphogenesis had been done prior to this investigation. Any changes in transcription would result in a change in mRNA species present throughout the life cycle. DNA-RNA hybridization is the most sensitive method presently known for comparison of RNA sequences (2). Competition experiments between RNA species synthesized at various times during morphogenesis were undertaken to determine uniqueness of messenger RNA from rods and spheres.

CHAPTER II

MATERIALS AND METHODS

Organism and Culture

For life cycle studies, Arthrobacter crystallopoietes (ATCC 15481) was grown in 500 ml TGY broth (0.5% (w/v) tryptone, 0.25% (w/v) yeast extract, 0.1% (w/v) glucose) in Erlenmeyer flasks at 30 C in a New Brunswick model VS rotary shaker at 175 rev/min. DNA was purified from cells grown in TGY broth for 20-24 hrs at 30 C in a 4 liter New Brunswick Microferm. Stock cultures were maintained on TGY agar plate and transferred at 48 hr intervals. Inoculum for broth cultures was prepared by transferring 48 hr cells to broth. Cultures for studies of morphogenesis were initiated with 3.5 ml of a 24 hr broth culture. Broth cultures prepared this way had an initial optical density of 0.04 at 475 nm on Spectronic 20 (Bausch and Lomb). Morphogenesis of Arthrobacter crystallopoietes was followed using the Webb cell wall stain (34).

Lysis Procedure

The following lysis procedure was modified from the method of J. Clark and G. Brownell (Nocardia Newsletter, No. 7, 1969).

Cells were washed 3 to 5 times with distilled water, then suspended in 0.15 M NaCl, 0.015 M sodium citrate buffer, pH 7.0 (hereafter abbreviated as 1XSSC), using approximately 20 ml buffer per gram wet packed weight of cells. Lysozyme (100 ug/ml) was added and the cell suspension inoculated at 37 C in a water bath for one hr. Pronase (100 ug/ml) was then added and 37 C incubation continued for one hr or until increased vicosity of the suspension was observed. A 25% (w/v) sodium lauryl sulfate solution was added to obtain final concentration of 50 ug/ml. The suspension was heated to 60 C for 5 to 30 min as necessary for lysis.

Enzymes

Ribonuclease (5X crystallized, Calbiochem) was heated to 95 C for 10 min, as a 1 mg/ml solution and stored at 4 C. The stock solution of pronase (1 mg/ml), (Calbiochem), was pre-incubated 1 hr at 37 C to destroy nuclease activity and stored at -10 C. Electrophoretically pure DNase was purchased from Worthington Biochemical.

Preparation of DNA Filters

DNA was purified from the cell lysate by Marmur's procedure (25) with intermediate enzymatic treatment with ribonculease (100 ug/ml) for an additional hr before final deprotienization. Denaturation of DNA and loading of DNA onto filters were carried out by the procedures of Gillespie and Spiegelman (18) except all DNA was loaded using 6XSSC as the loading solution. One or two $O.D._{260}$ of native DNA were loaded on each 25 mm nitrocellulose filters (Scheicher and Schuell, type B6). The amount of DNA on sample filters was determined by the procedure of Bonner et al. (6).

Purification of RNA

RNA was purified from cell lysates by extraction with water saturated phenol and precipitation from 70% (w/v) ethanol. 1/10XSSC plus 0.01M $MgCl_2$ was used for RNA purification. After 3 or 4 phenol extractions, RNA was treated with DNase (50 ug/ml) for 1 hr at 37 C, followed by pronase (100 ug/ml) for an additional hr at 37 C. Phenol extraction was continued until almost no protein appeared at the phenol-water interface. RNA was precipitated with 2 volumes 95% ethanol and resuspended in 0.05M potassium phosphate buffer, pH 6.8, in preparation for hydroxyapatite chromatography.

Hydroxyapatite Column Chromatography of RNA

Three gm of Bio-Gel HTP (Bio-Rad Laboratory, Richmond, California), a hydroxyapatite gel, was washed with

three 40 ml portions of 0.05 M potassium phosphate buffer, pH 6.8 and the fines decanted. A slurry of the hydroxyapatite in 50 ml of the same buffer was packed in a 9 mm Sephadex column fitted with a glass wool filter. The void volume and uniformity of bed packing were determined by observing the passing of methyl orange through the column. The column was washed with 40 ml of the packing buffer before use.

Samples containing up to 3 mg of RNA, which had been phenol extracted and enzymatically treated were loaded on the column in 1 to 2 ml of the packing buffer. RNA was eluted with a linear gradient of 0.05--0.3 M potassium phosphate, pH 6.8, with a total volume of 140 ml. Protein and nucleic acid elution was followed by UV absorption at 254 nm and 2.5 ml fractions collected in an Isco Ultraviolet Analyzer, Model UA-2, and fraction collector. RNA elution was carried out at 4 C with an initial column pressure of 80 g/cm^2. Fractions containing RNA were pooled, precipitated in 70% (w/v) ethanol and resuspended in 1XSSC for hybridization.

The hydroxyapatite column was washed with 40 ml of 0.4M potassium phosphate buffer, pH 6.8, followed by 40 ml of packing buffer, before re-use. Three or four samples were run on each column prepared.

Sucrose Gradients

One-tenth ml of 1XSSC containing 25-80 ug of RNA was layered on 4.2 ml of a 5 - 20 percent sucrose gradient in 1XSSC and centrifuged 2 1/2 hrs at 10 C at 65,000 r.p.m. (42,000 x G) in the SW 65 Ti head in a Beckman L2-65B ultracentrifuge. Gradients were read at 254 nm through an Isco upward flow cell. Four drop fractions were collected and counted when labeled RNA was analysed.

DNA-RNA Hybridization

Initial hybridizations were done using the high temperature method of Gillespie and Spiegelman (18). Later hybridizations, including all saturation and competition curves, were done at room temperature (24-25 C) incorporating 30% (v/v) formamide as described by Bonner et al. (6). One or two ml volumes were used for all hybridizations. Blank filters were washed by suction filtration with 50 ml of 6XSSC of each side and heated 2 hrs at 80 C in a vacuum oven. All hybridizations were run 12 hrs with duplicate DNA containing filters. For competition experiments, varying amounts of unlabeled RNA were hybridized, and the filters incubated with 20 ug/ml ribonuclease, followed by a second hybridization with P^{32} labeled RNA and ribonuclease treatment.

Nucleic acid concentration was determined spectrophotometrically assuming coefficients of 2.3×10^4 cm^2/gm for RNA and 2.5×10^4 cm^2/gm for DNA at 260 nm.

Radioactivity Determination

RNA was labeled by addition of P^{32} orthophosphate (150 uCi/500 ml) during the last 2 hrs of growth. Some cultures for extraction of DNA were labeled with H^3-thymidine (30 uCi/l). Radioisotopes and Omnifluor were purchased from New England Nuclear, Boston, Massachusetts.

Samples were counted in 10 ml of dioxane containing 100 g/l naphthalene and 8 g/l Omnifluor in a Packard Tri-Carb liquid scintillation counter.

CHAPTER III

Results

The maximum changes in cell morphology during sphere-rod morphogenesis of Arthrobacter crystallopoietes are expressed during growth in a complex medium such as tryptone-yeast extract-glucose broth (Fig. 1). Under the conditions of growth described in Materials and Methods, stationary spherical cells transferred to fresh broth will germinate into rods. First division of the rods occurs between 4 and 5 hrs. Greatest rod length occurs between 10 and 12 hrs. The rods shorten after 12 hrs, a process which has been called fragmentation. Cell wall stains reveal many cells containing three cross-septa, and both direct cell counts and plate counts of cultures show a 4-fold increase in cell number at the onset of rod-shortening. This suggests that fragmentation may be two closely spaced binary divisions. The 3 ages chosen for study were 4 hr - first rod division, 10 hr- mid-log phase and greatest rod length, and 16 hr - late log phase shortening rods.

High molecular weight DNA from Arthrobacter crystallopoietes was needed for DNA-RNA hybridization experiments. Neither osmotic shock nor treatment with lysozyme was

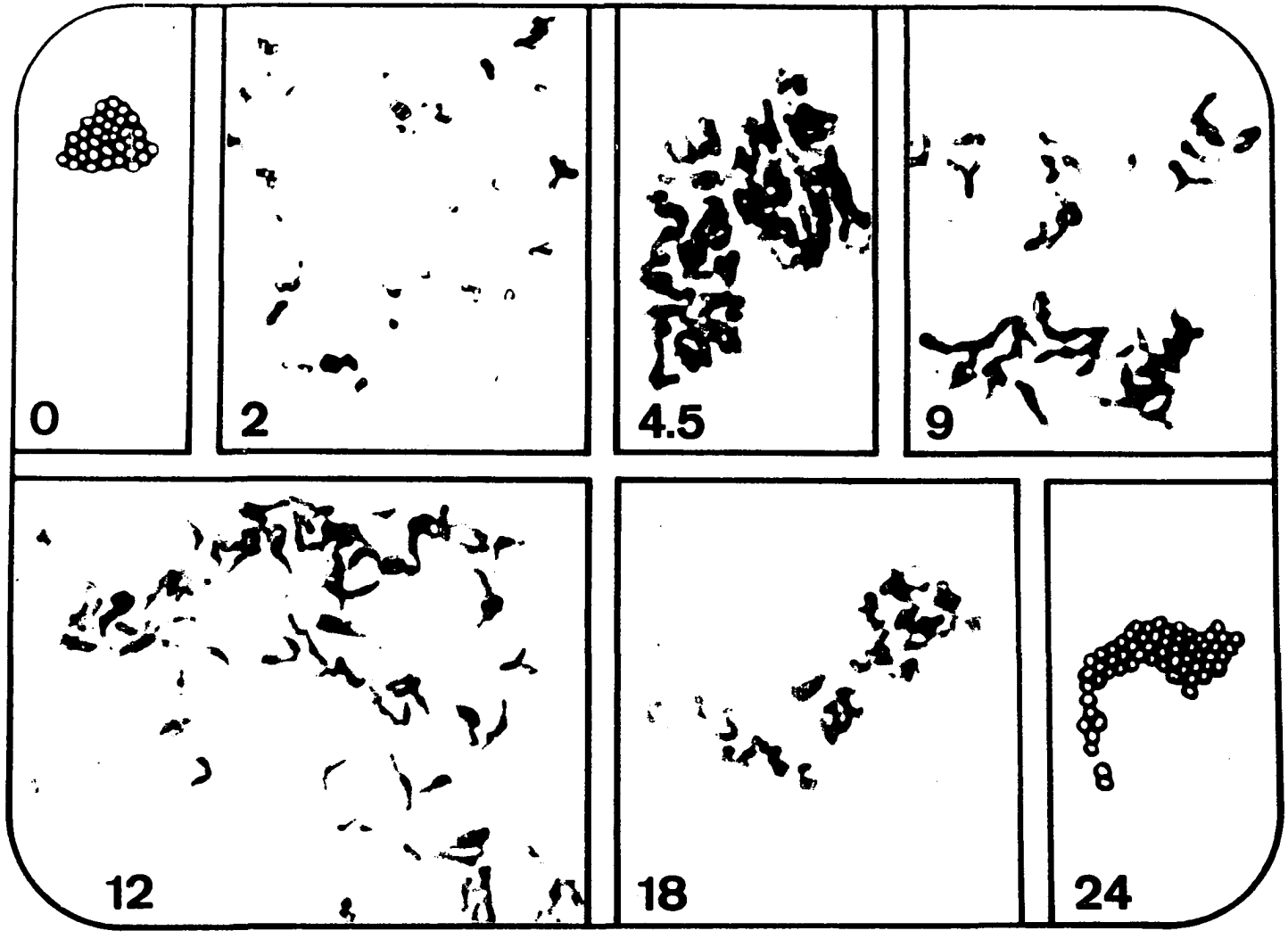

Fig. 1--Life cycle of Arthrobacter crystallopoietes in TGY broth. Culture as described in Materials and Methods. Cell walls were stained with the Webb cell wall stain. Numbers show age of culture in hours.

efficient in lysing cells. Cells of Arthrobacter crystallopoietes showed varying resistance to lysis with lysozyme as a function of culture age (Fig. 2). Rods had greater resistance to lysozyme than spheres. The 48 hr spheres used as inoculum had an extracellular slime layer which was not removed during washing and which apparently prevented lysozyme from contacting the cell walls. The 24 hr spheres had synthesized very little of the protecting slime layer and 90% were sensitive to lysozyme. However, cells pre-treated with lysozyme could be lysed by incubation with pronase followed by the addition of sodium lauryl sulfate.

Hybridizations were carried out with DNA bound to filters. Binding the DNA to the nitrocellulose filters eliminates DNA renaturation during hybridization (17). However, the use of filters introduces the problem of high levels of "noise," i.e., non-specific binding of RNA to the filters. Most noise results from basic proteins present in RNA or DNA which bind to both RNA and the membrane filters. Preliminary experiments of this study were run to determine conditions which minimized noise.

Noise levels were unaffected by increasing ribonuclease concentration to 40 ug/ml or by incubating with pronase at 50 ug/ml after ribonuclease treatment. Increasing the salt concentration from 2XSSC to 6XSSC increased noise. Some reduction of noise was achieved by washing blank filters with 50 ml of 6XSSC on each side and heating as for

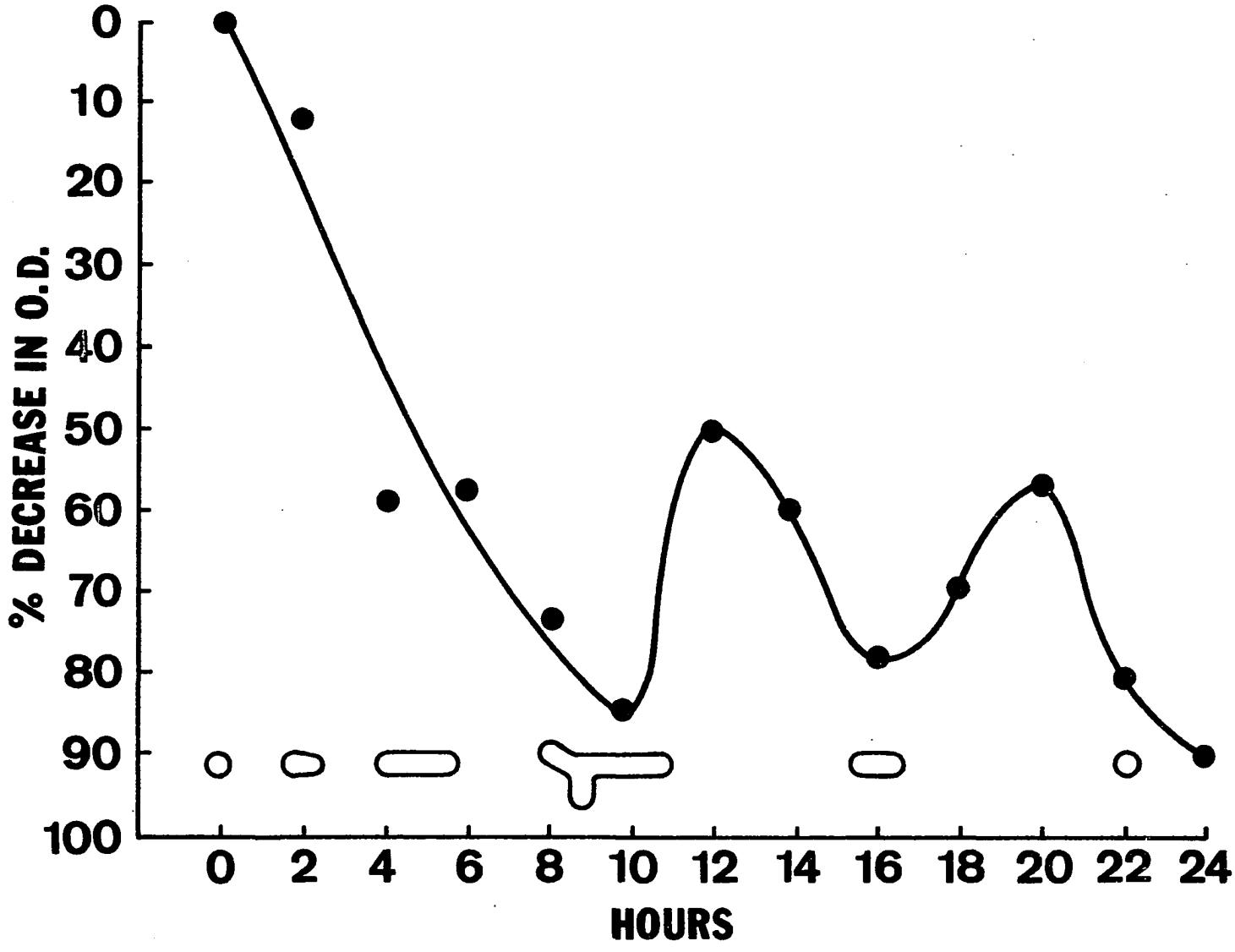

Fig. 2.--Sensitivity of cells to lysozyme as a function of culture age. Lysozyme concentration was 1.0 mg/ml in water.

DNA-containing filters. The most effective method for reduction of noise was further purification of RNA preparations by elution from a hydroxyapatite column with a 0.05M-0.30M potassium phosphate gradient. Purification by hydroxyapatite chromatography decreased noise of input RNA from 2.5-3.2% to 0.1-0.3%.

Solutions containing RNA and various compounds which might be expected to interfere with hybridization were prepared and analyzed by hydroxyapatite chromatography (Fig. 3). Phosphate molarities of certain fractions were calculated from the proportion of gradient previously collected to the total volume used for the gradient (140 ml) after correction for the void volume (10 ml). The absorbance of phenol at 260 nm would interfere with spectrophotometric determination of RNA concentration and give erroneous saturation and competition values. In Fig. 3a, it can be seen that phenol passed through the column without binding (4). Unless large amounts of phenol were present in the RNA loaded onto the column, phenol could easily be separated from RNA. The separation of lysozyme and pronase from RNA was also examined (Fig. 3b). Gillespie and Spiegelman (18) found 20 ug/ml lysozyme could bind 74.9% of input RNA. Both pronase and lysozyme were eluted soon after the void volume, while tRNA was eluted at 0.11 M phosphate. The second peak in Fig. 3c was ribosomal RNA and eluted at 0.13 N phosphate.

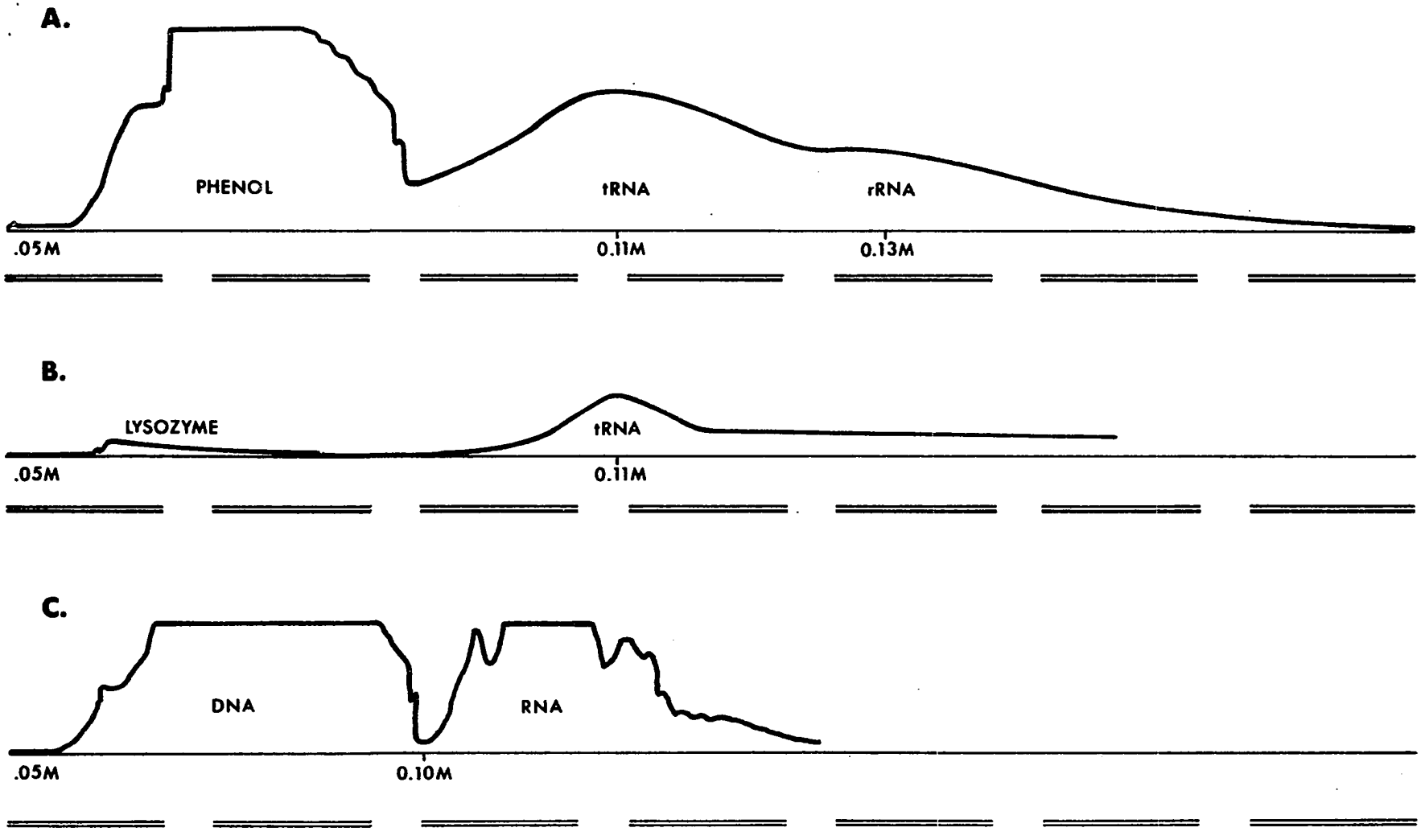

Fig. 3.--Chromatography of RNA on a hydroxyapatite column. A. Arthrobacter RNA without removal of phenol. B. One mg E. coli t RNA plus 10 mg lysozyme. C. Arthrobacter RNA plus DNA, incubated with DNase as described in Materials and Methods.

DNA in RNA preparations will compete with DNA bound on the filter and contribute to noise, so separation of DNA from RNA is also desired. In Fig. 3c Arthrobacter RNA and the oligodeoxynucleotides resulting from DNase digestion can be seen to elute at distinctly different phosphate molarities. Several fractions in each peak were hydrolyzed in 0.3 M KOH overnight at 24 C, precipitated with 5% perchloric acid and absorbance of the supernatant at 260 nm determined. The first peak eluted was alkali stable, while the second peak was hydrolyzed by the base.

The efficiency of hydroxyapatite in separating RNA from contaminating compounds permitted the omission of several phenol extractions after enzymatic treatment. Calculations of yield of RNA eluted from the column was near 100% for runs analyzed. No significant amount of material absorbing at 254 nm was eluted during the 0.4 M potassium phosphate wash after RNA chromatography.

Fig. 4 shows sucrose gradient analysis of P^{32} labeling and molecular weight distribution of two RNA preparations. The 10 hr short-labeled RNA preparation shows labeling intermediate to size to 5s and 16s rRNA, indicating the presence of some mRNA. Long-labeled RNA shows little intermediate sized labeled RNA. Sucrose gradients of total RNA samples before and pooled RNA fractions after hydroxyapatite chromatography revealed no changes in molecular weight of RNA. Lack of breaks, 100% yields, and the

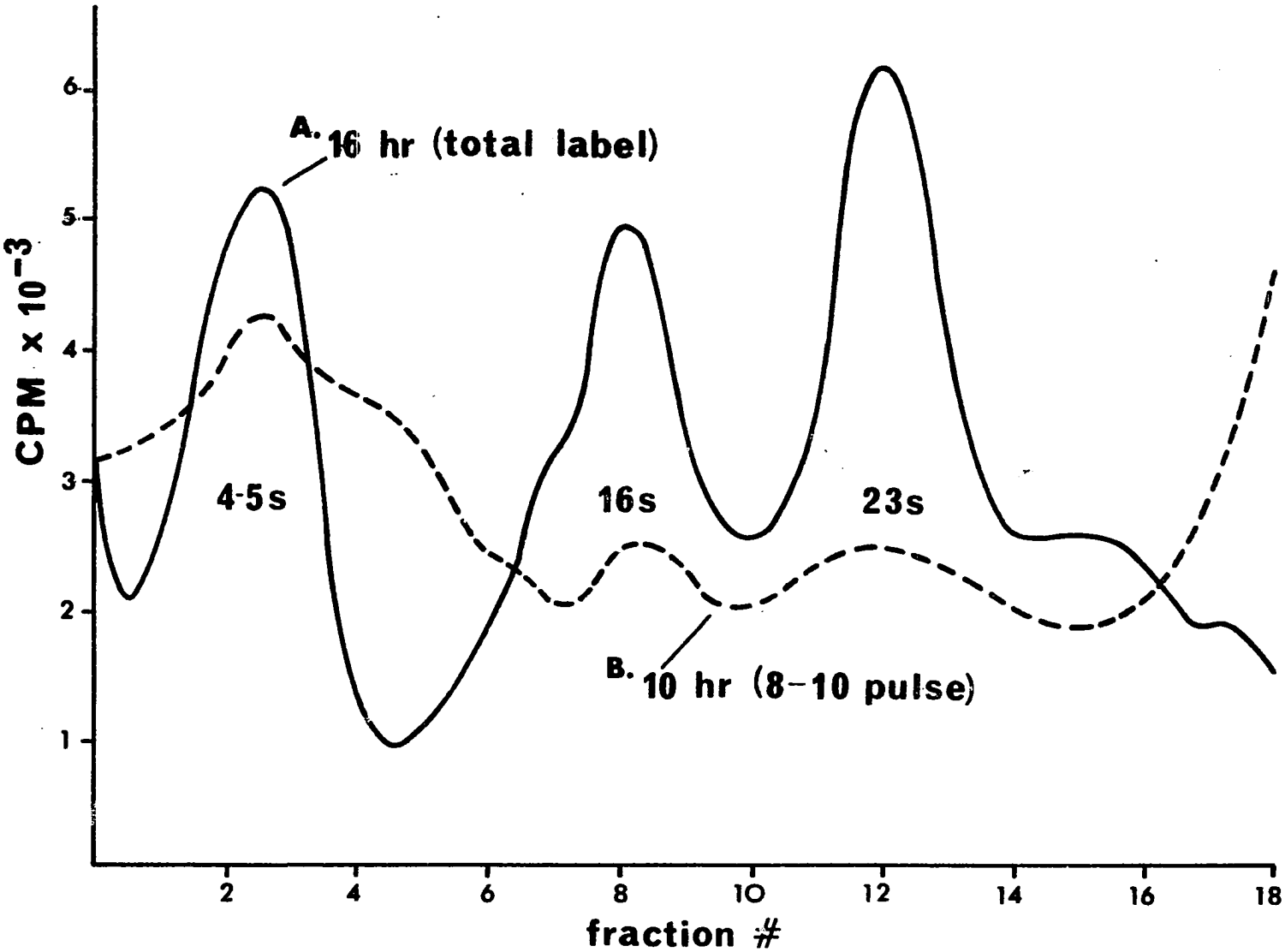

Fig. 4.--Incorporation of P^{32} orthophosphate in Arthrobacter RNA. A. Cells labeled throughout 16 hr growth period. B. Cells labeled last two hrs of 10 hr growth period.

phosphate molarity required for elution of various compounds agreed with results published by Bernardi (5). There was only minor absorption at phosphate molarities below 0.10 M in RNA preparations used in hybridization, indicating little contamination with phenol and oligodeoxynucleotides even before hydroxyapatite chromatography.

Initial hybridizations concerned with noise reduction were conducted at 66 C (18). Later hybridizations were done at 24-25 C in the presence of 30% (v/v) formamide (6). Substitution of 30% formamide for high temperature gives the same degree of renaturation of the nucleic acids (27) with the reduction of thermal degradation of RNA. Use of formamide also reduces loss of DNA from filters during hybridizations (6). Sample filters from the same lot containing tritium labeled DNA retained 977 counts/min/filter during formamide hybridization, while filters subjected to high temperature hybridization retained only 838 counts/min/filter.

Saturation curves for RNA preparation for each of the three ages are presented in Fig. 5. The amount of DNA hybridizing to RNA rises with increased RNA input. No plateaus are observed on any saturation curves so percentage saturation is reported as the value obtained with the highest RNA input used. The values for saturation were found to be 1.60%, 2.14% and 1.18%, respectively, for 4 hr, 10 hr, and 16 hr RNA.

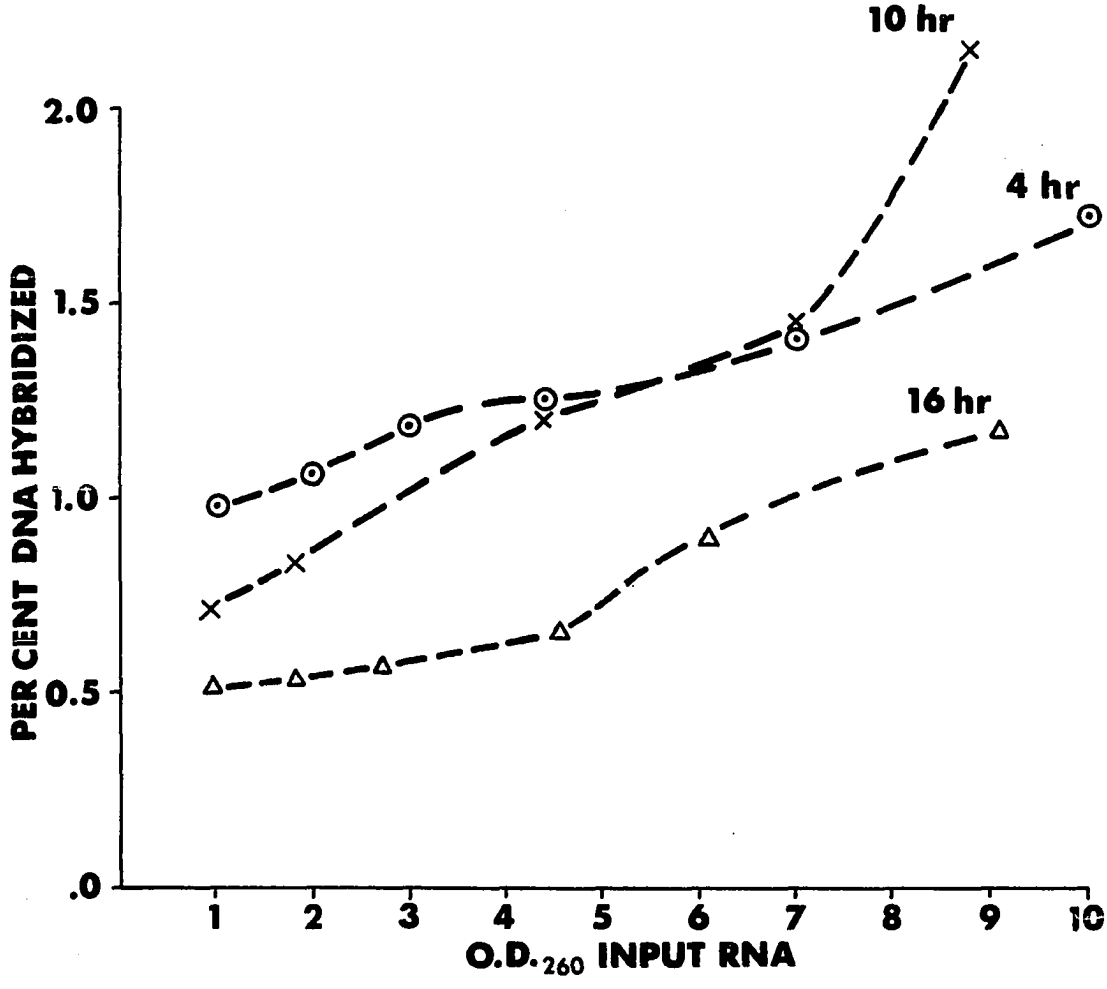

Fig. 5.--RNA saturation curves from Arthrobacter crystallopoietes at various ages. Filters contained 80 ug DNA.

All three RNA saturation curves have a change in slope near a RNA input of 4 to 5 $O.D._{260}$. These indicate the presence of two major groups of RNA which vary significantly in concentration. The first three or four points on each saturation curve represent the saturation of DNA sites with RNA species present in higher concentration. These points can be used to calculate the percent of DNA saturated by these high concentration species. Saturation of the DNA by these RNA species follows first order kinetics, so a double reciprocal plot of the values in Fig. 5 should be linear. A line was fitted to the points obtained using the least squares equation. Extrapolation of this line to zero RNA input showed that the high concentration RNA species from 4 hr cultures saturated 0.62% of the DNA, RNA from 10 hr, 0.87% and RNA from 16 hr, 0.48%.

Competition experiments were done to obtain information about differences in RNA species present at various culture ages. The results of sequential competitive hybridizations are shown in Figs. 6, 7 and 8, and summarized in Table 1. Prior hybridization with unlabeled RNA prevents labeled RNA species which have the same sequence from hybridizing, since complementary DNA sequences are blocked. Labeled RNA sequences not present during the first hybridization account for hybrids formed during the second hybridization.

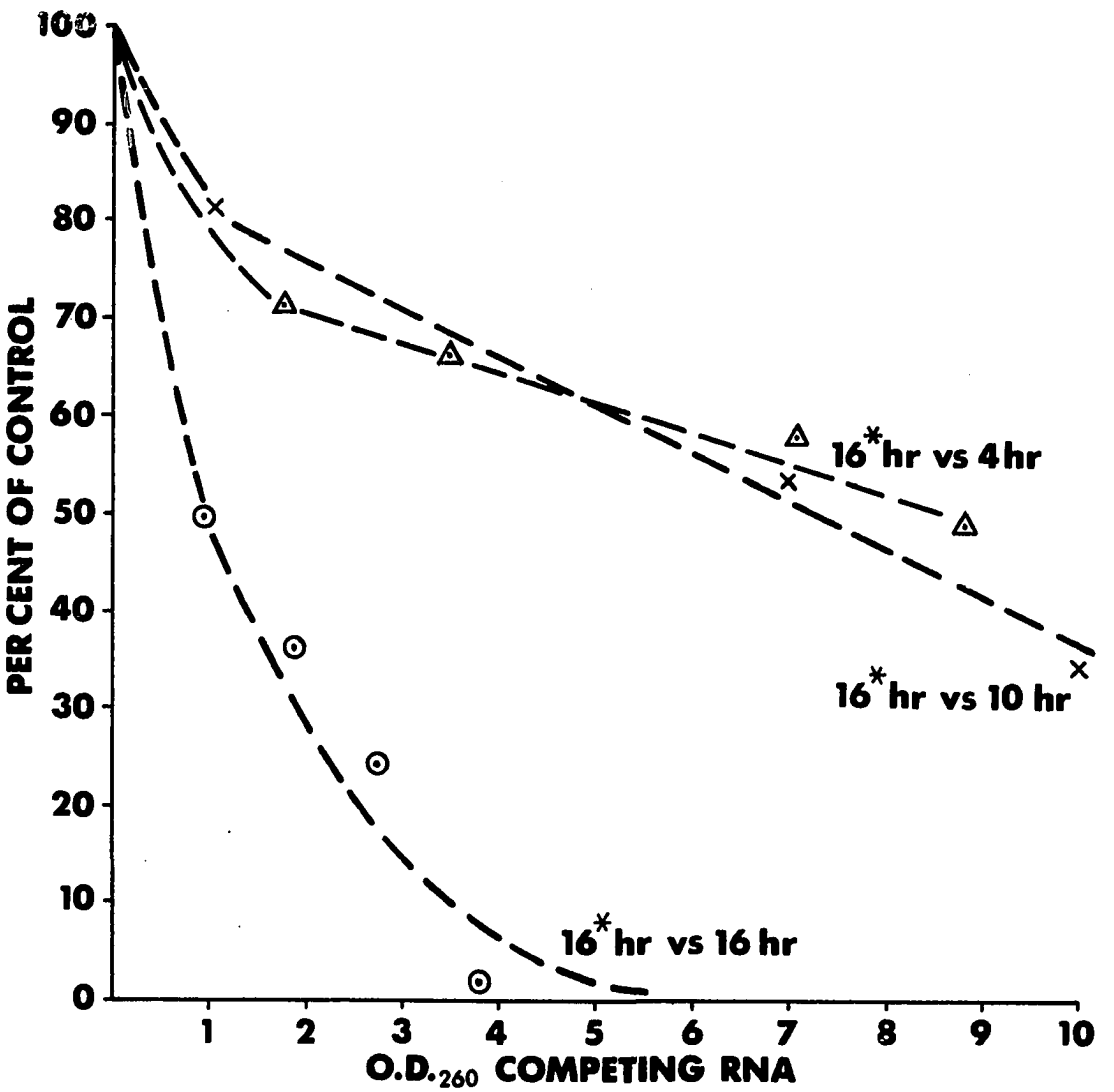

Fig. 6.--Competition experiments with 16 hr P^{32} labeled RNA, 9.1 $O.D._{260}$/ml. Varying amounts of unlabeled RNA from 16 hr, 10 hr, and 4 hr cells were prehybridized as indicated. P^{32} labeled RNA designated by *.

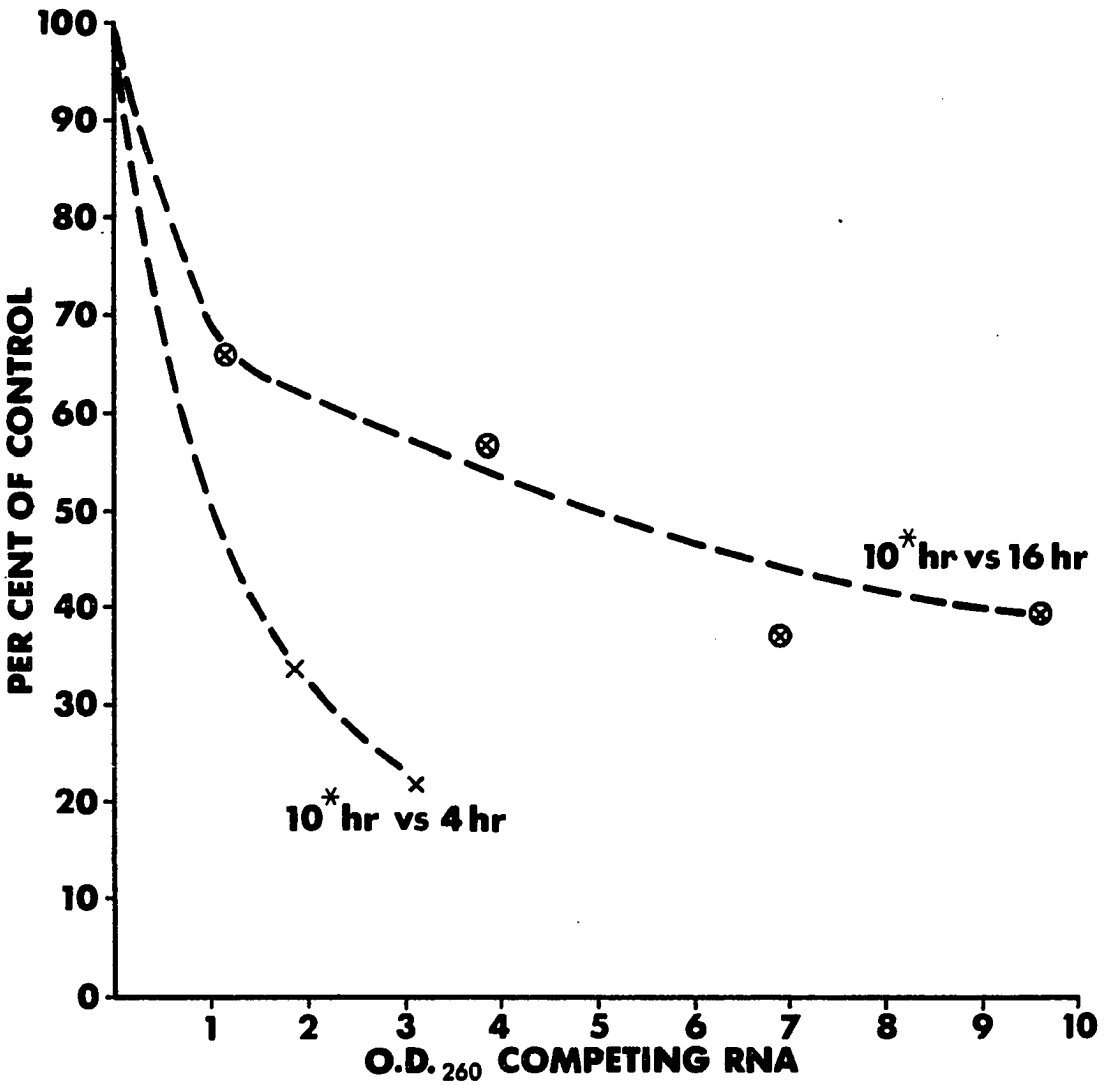

Fig. 7.--Competition experiments with 10 hr P^{32} labeled RNA, 8.61 $O.D._{260}$/ml. Varying amounts of unlabeled RNA from 16 hr and 4 hr cells were pre-hybridized as indicated. P^{32} labeled RNA designated by * .

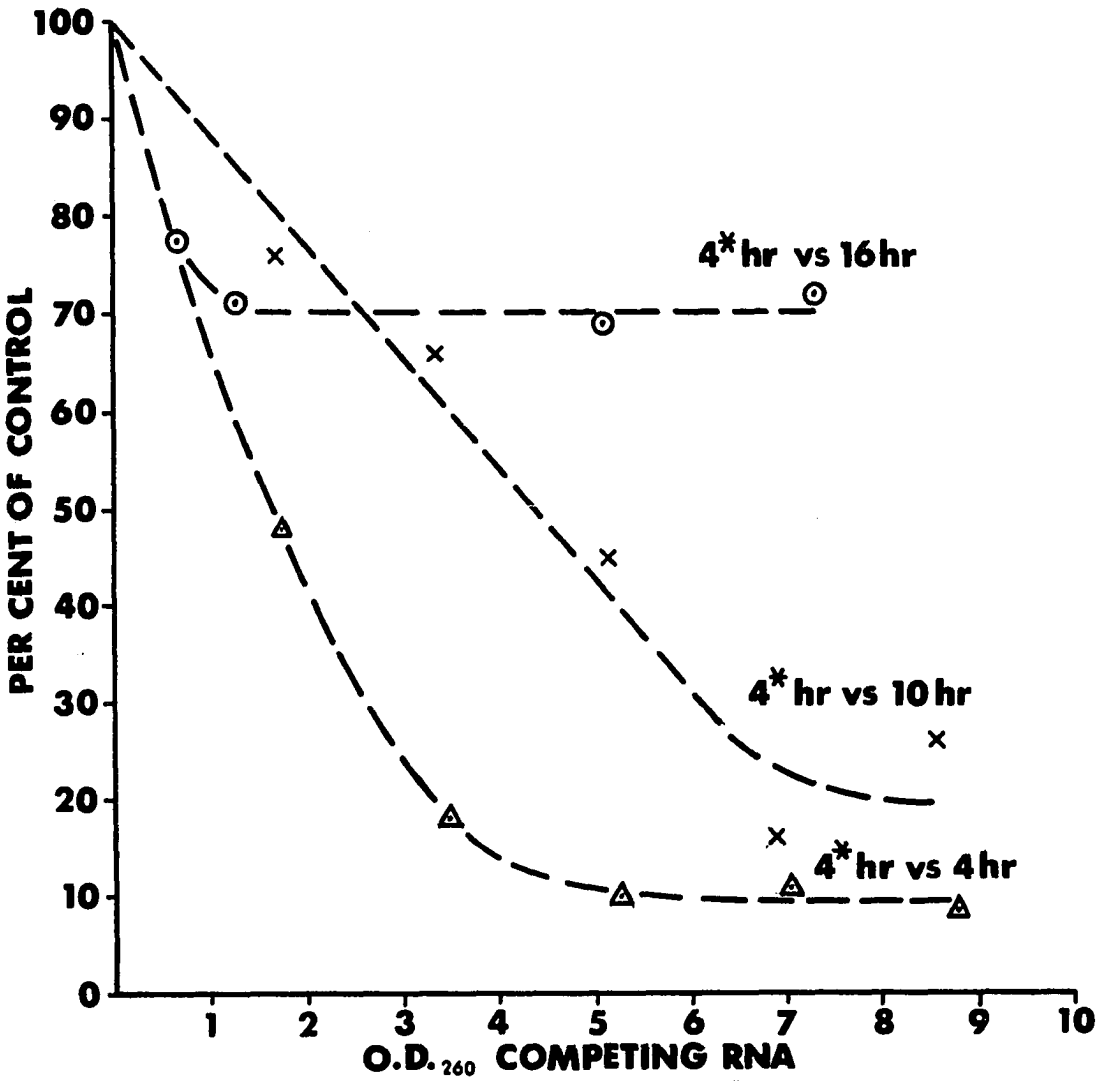

Fig. 8.--Competition experiments with 4 hr P^{32} labeled RNA, 8.60 $O.D._{260}$/ml. Varying amounts of unlabeled RNA from 16 hr, 10 hr and 4 hr cells were prehybridized as indicated. P^{32} labeled RNA designated by *.

TABLE 1

SUMMARY OF COMPETITION EXPERIMENTS

P^{32} Labeled RNA	Unlabeled RNA		
	4 hr	10 hr	16 hr
4 hr	91%	79%	28%
10 hr	80%	--*	66%
16 hr	49%	60%	99%

*Not done.

CHAPTER IV

DISCUSSION

During development of a lysis procedure, cells of Arthrobacter crystallopoietes were shown to vary in sensitivity to lysozyme as a function of culture age, with rods more resistant than spheres. Krulwich et al. (20) have reported that the cell wall of rods contains polysaccaride backbones three times the length of those in spheres. Cleavage of the glycosidic bonds by lysozyme in a sphere wall containing only short polysaccharides might weaken a sphere cell wall considerably more than a rod cell wall which contains the longer polysaccharides. Spheres would thus be more sensitive to lysozyme. Krulwich and Ensign (22) found higher specific activity of an autolytic N-acetyl-muramidase in sphere walls than in rod walls. They suggested that the enzyme might be causally related to cell morphogenesis. It was hypothesized that the spherical shape results from a less rigid cell wall, due to shorter polysaccharides and longer peptide cross-bridges.

Initial hybridizations had a noise level so high it often obscured counts due to hybridized RNA. Preliminary

experiments were run to determine the conditions of hybridization which minimized noise. From these experiments the optimum conditions were found to be incubation in 2XSSC for 12 hr, using blank filters washed with 6XSSC and heated 2 hr at 80 C, with high temperature (66 C) as the renaturing agent. For determination of saturation and competition values, 30% formamide was substituted as the renaturing agent. McConaughy et al. (27) showed renaturation with 66 C and 30% formamide to be equivalent in 2XSSC.

The most effective way of reducing noise was purification of the phenol-extracted and enzymatically treated RNA on a hydroxyapatite column. Elution with a linear gradient of 0.05 M - 0.30 M phosphate, pH 6.8, separated RNA from contaminating protein, phenol and oligonucleotides. Adsorption of RNA occurs because of the interaction between negatively charged phosphate groups of RNA and the positive calcium ions on the surface of the hydroxyapatite crystals. Basic proteins, which cause high levels of noise when present in RNA or DNA, are retained less strongly than polynucleotides, since the carboxy groups of these proteins have a lesser affinity for calcium ions than phosphate groups. Elution of polynucleotides from hydroxyapatite is caused by the progressive increase of the molarity of the eluting phosphate buffer and is due to the specific competition between the phosphate ions of the eluting phosphate buffer and the phosphate groups of polynucleotides for calcium ions of

hydroxyapatite. The elution order of polynucleotides from hydroxyapatite is dependent on the negative charge density of the polynucleotides. Flexible randomly-coiled polynucleotides are eluted from hydroxyapatite by lower phosphate molarities than rigid, helical polynucleotides. Britten and Kohne (7) have used this principle to separate single-stranded DNA from renatured hybrids after liquid hybridization.

The saturation values of Arthrobacter total RNA was found to vary as a function of culture age, with the greatest portion of the DNA being transcribed in 10 hr rod cells. The most abundant messages will saturate their gene sites first. With increasing RNA input less abundant species will saturate their complementary DNA. Cistrons for rRNA and tRNA have been shown to account for 0.35% of the DNA in E. coli (19). In the same paper Kennell calculated that 80% of E. coli RNA was rRNA and 17% was tRNA, and these stable RNA species saturated their gene sites at a 1/160 RNA/DNA ratio. Ribosomal and transfer RNA are present in great excess even at the lowest RNA input used in these experiments and will account for a constant percent of the RNA hybridized.

Since the % saturation for RNA species present in high concentration excedes that expected for rRNA and tRNA, some mRNA species must be relatively abundant. Among these messages probably would be messages for DNA and RNA polymerases, ribosomal proteins, tRNA acylating enzymes and

tricarboxy acid cycle enzymes. These messages, tRNA and rRNA should be transcribed throughout growth regardless of the stage of sphere-rod morphogenesis. The difference between total saturation and the saturation values determined for tRNA, rRNA and the omnipresent messages will mostly reflect changes in transcription during sphere-rod morphogenesis. In elongating rods in 4 hr cultures, the messages being transcribed from the remaining 0.73% of the DNA direct synthesis of enzymes unique to rod growth. This 0.73% will be arbitrarily called "rod messages." Similarly in 16 hr cultures the 0.70% remaining will be called "sphere messages." If it is assumed that 10 hr rods are a transition state between rods and spheres, with both rod messages and sphere messages being transcribed in addition to omnipresent messages, 2.05% of the DNA should be hybridized, which is close to the value of 2.14% determined by saturation experiments.

With the assumption that 10 hr rods contain both rod and sphere messages, expected competition values were calculated from the values in Table 2. A comparison of theoretical and experimental values is presented in Table 3. There is good agreement between theoretical and experimental values. In calculating theoretical competition, it was assumed no rod messages were present at 16 hr and no sphere messages were present at 4 hr.

TABLE 2

THEORETICAL CONTRIBUTION OF RNA COMPONENTS TO SATURATION VALUES

	4 hr	10 hr	16 hr
% DNA Transcribed	1.60%	2.14%	1.18%
rRNA, tRNA and omnipresent concentration messages*	0.87%	0.62%	0.48%
"Sphere" messages		0.70%	0.70%
"Rod" messages	0.73%	0.73%	
	1.60%	2.05%	1.18%

*Calculated from data in Fig. 5.

TABLE 3

COMPARISON OF THEORETICAL AND EXPERIMENTAL COMPETITION VALUES

Unlabeled RNA	Labeled RNA	Theoretical Competition	Experimental Competition
4 hr	4 hr	100%	100%*
10 hr	4 hr	100%	87%*
16 hr	4 hr	30%	31%*
4 hr	10 hr	67%	80%
16 hr	10 hr	59%	66%
4 hr	16 hr	44%	49%
10 hr	16 hr	100%	60%
16 hr	16 hr	100%	99%

*Calculated from Table 1. The experimental level of self-competition of 4 hr RNA is set at 100%, and the values observed for the other hybridizations with 4 hr labeled RNA adjusted proportionately.

The same arguments would be equally valid if rod messages were called early log messages and sphere messages termed late log messages. Very little experimental work has been done on aging in bacterial cultures and it is not known how greatly transcription changes due to aging of a culture.

It is reasonable to assume that 10 hr rods contain both rod and sphere messages, even though all cells are rods at their greatest length. Cross-septa for the first division which results in shorter rods are first visible at 12 hr with the Webb cell wall stain. The two hour interval represents only 1 1/3 generations of growth of Arthrobacter in TGY broth. Transcription of sphere genes must occur earlier than 12 hr for expression of those genes at 12 hr. Aronson (1) reported that enzymes for sporulation of Bacillus cereus appeared after 8.5 to 9 hrs of growth, while the forespores were not visible until after 14 to 15 hrs of growth.

The competitive hybridization experiments show that messenger RNA populations change considerably when comparing elongating and shortening rods, with 10 hr rods as a transition state. One mechanism which could account for differential transcription is the synthesis of a new species of RNA polymerase with different specificity. This has been shown to be the case during T7 phage infection (9). Another mechanism is the alteration of the existing RNA polymerase. A subunit of RNA polymerase molecule called the σ factor was

shown to be responsible for the specificity of transcription which RNA polymerase exhibits (3,8,11,30,31,32,33). During endospore formation, differential synthesis of messenger RNA was demonstrated (1,13). The template specificity of RNA polymerase was shown to change during sporulation of Bacillus subtilis (24). Since a rifampicin-sensitive component of RNA polymerase was conserved during sporulation, it was postulated that the σ factor was involved in the change of template specificity. Similar results were obtained by Okana et al. (28) who found changing transcription during microcyst formation in Myxococcus xanthus.

CHAPTER V

SUMMARY

The sphere-rod morphogenesis of Arthrobacter crystallopoietes has several advantages as a system for studying bacterial control mechanisms. The morphogenic cycle is relatively simple, quantitative, synchronous and can be nutritionally controlled.

Several recent investigations on morphogenesis in A. crystallopoietes describe changes in enzyme levels which correlate with morphological changes, and some work has been reported on enzyme level control. No work on control at the level of transcription has been previously reported.

During development of a lysis procedure, variation in sensitivity to lysozyme during the life cycle was found. The change in sensitivity can be correlated with changes in length of cell wall polysaccharide backbones.

Initial membrane-bound DNA-RNA hybridization had high levels of nonspecific RNA binding, or "noise." Optimum conditions for reduction of noise were found to be incubation for 12 hr in 2XSSC at 66 C using blank filters which had been washed and heated. Purification of RNA on a

hydroxyapatite column further decreased noise by removal of contaminating proteins, phenol and oligonucleotides.

Hybridization experiments were done using procedures for lysis and RNA purification on hydroxyapatite which had been developed. Saturation levels of RNA were shown to vary with culture age, with the greatest portion of DNA being transcribed at 10 hr. Calculations of levels of competition expected between ages correspond to experimental values, if some unique messages are found in rods and sphere, and assuming 10 hr rods are in a transition stage between rods and spheres, and contain both rod and sphere messages.

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