Nucleic Acid Synthesis during the Vegetative Life Cycle of *Volvox aureus* M5

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**Summary.** 1. The synthesis of both DNA and RNA are shown to follow the same general pattern during the vegetative life cycle of *Volvox aureus* M5. Nucleic acid synthesis is minimal during the first 12 h of development and maximal at the 36–48 h phase.

2. Mitomycin C at 45 μg/ml and actinomycin D at 4 μg/ml are shown to selectively inhibit DNA and RNA synthesis respectively.

3. The continued synthesis of DNA and RNA throughout the period of cell division is required for normal completion of development. Gonidial enlargement is not inhibited by either antibiotic.

*Volvox aureus* is a green colonial flagellate consisting of 500–1000 biflagellate cells embedded in a common colonial matrix. *Volvox* is an excellent organism for developmental studies because of its simplicity and because of chemical systems known to control various aspects of its development (Darden, 1966, 1970; Starr, 1970). Although the biochemical and molecular mechanisms involved in the differentiation of *Volvox* are unknown, it is to be expected that nucleic acids are intimately involved. Therefore, investigation of nucleic acid synthesis in *Volvox* should lead to a further understanding of differentiation and development in this organism. The only studies of *Volvox* nucleic acids have been those of Kochert (1971) and Kochert and Sansing (1971) on the isolation and characterization of nucleic acids from *Volvox carteri*. There is no information about the course of nucleic acid synthesis throughout the complete developmental cycle.

The current work was undertaken to determine the pattern of nucleic acid synthesis during the normal vegetative life cycle of *Volvox aureus* M5 and to study the effects of the inhibition of DNA and RNA synthesis at various stages of development.

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Material and Methods

Organism and Culture. *Volvox aureus* used in this investigation was the homothallic, dioecious M5 strain (Darden, 1966). Organisms were grown axenically in *Volvox* medium (Provasoli and Pintner, 1959) at 22 ± 3°C. Continuous illumination with an intensity of approximately 600 ft-c was provided by cool white fluorescent tubes.

Preparation of Synchronized *Volvox*. Parent colonies, selected just prior to the release of young, were placed in Petri dishes containing fresh *Volvox* medium and allowed to release offspring. These young colonies tend to develop synchronously through at least one generation. This method was used to provide large pools of synchronized colonies which were used in all other experiments.

Preparation of Isotopically Labeled Nucleic Acids. Nucleic acids were labeled with thymidine-methyl-³H (specific activity: 19.9 Ci/mmole) and uridine-2-¹⁴C (specific activity: 55.6 Ci/mmole) purchased from New England Nuclear, Boston, Massachusetts. Triated thymidine was used at a final concentration of 5 μCi/ml and uridine-2-¹⁴C at 0.1 μCi/ml.

Beginning with newly released colonies, 1000 vegetative coenobia in 5.0 ml of medium were added to 5.0 ml of a mixture of thymidine-methyl-³H and uridine-2-¹⁴C. At 12-h intervals, up to a total of 48 h, an additional 1000 colonies from a synchronized pool were placed in other dishes containing isotopes. Colonies from each developmental stage were allowed to remain in isotopes for 12 h after which the colonies were removed by Millipore filtration and washed with 20 ml of deionized water.

Nucleic Acid Extraction. Total nucleic acids were extracted by a modification of the phenol procedure used by Kochert and Sansing (1971) for *Volvox carteri*. In these experiments, 1000 colonies containing isotopically labeled nucleic acids were homogenized in 5.0 ml of TNSB buffer (0.1 M Tris pH 8.0 + 0.1 M NaCl + 2% (w/v) recrystallized sodium dodecylsulfate + 1 mg/ml bentonite). Homogenization was for 3 min at room temperature in a Micro-mill (Chemical Rubber Company) attached to a Waring blender. The homogenate was rinsed from the Micro-mill with an additional 5.0 ml of TNSB buffer and 10 ml of phenol reagent added (500 g redistilled phenol + 70 ml redistilled m-cresol + 0.5 g 8-hydroxyquinoline + 55 ml deionized water). This mixture was magnetically stirred for 15 min, cooled in ice and centrifuged at 5°C. Centrifugation was for 15 min at 6780 x g in a SS-34 rotor of a Sorvall RC-2B centrifuge. The upper aqueous phase was removed and an additional 10 ml of TNSB buffer added to the phenol phase. This mixture was stirred and centrifuged as before. The second aqueous phase was removed and combined with the first. The combined phases were again extracted with phenol. One mg of unlabeled carrier RNA (Sigma Chemical Company sRNA type III) and 0.05 vol of 3 M potassium acetate were added to the final aqueous phase. Nucleic acids were precipitated with 1.5 vol of cold 95% (v/v) ethanol for 12 h at −20°C. The final precipitate was collected by centrifugation, washed twice with cold 80% (v/v) ethanol containing 0.01 M Tris pH 8.0 and dried in a calcium chloride desiccator at −20°C.

Determination of DNA and RNA Content. The isotopically labeled nucleic acids were digested with DNase and RNase. The ³H counts solubilized by DNase digestion and the ¹⁴C counts solubilized by RNase digestion were considered to be specifically contained in DNA and RNA respectively. DNase (Deoxyribonuclease I, electrophoretically purified, Worthington Chemical Corporation) and RNase (Ribonuclease A Type III from bovine pancreas, Sigma Chemical Company) were prepared in acetate buffer (225 ml deionized water containing 12.5 ml 0.1 M MgSO₄ + 25 ml of 1.0 M acetate buffer pH 5.0). Nucleases were checked for purity prior to use. The nucleic acid pellet, prepared as above from 1000 colonies, was dissolved in 3.0 ml of