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Aphidicolin Inhibits Growth and DNA Synthesis in Halophilic Archaebacteria

PATRICK FORTERRE,* CHRISTIANE ELIE, AND MASAMICHI KOHIYAMA

Institut Jacques Monod, Université Paris VII, 75251 Paris Cedex 05, France

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Aphidicolin, a specific inhibitor of eucaryotic α DNA polymerase, inhibits the growth of halophilic archaebacteria. In *Halobacterium halobium*, aphidicolin prevents cell division and DNA synthesis. These results suggest that archaebacterial replicases are of the eucaryotic type.

Archaebacteria, eubacteria, and eucaryotes represent three distinct lines of cellular descent which diverged early in evolution (11). Archaebacteria are procaryotes in terms of cellular organization, but many details of their molecular biology resemble those of eucaryotes (3–6). Up to now, research on archaebacterial molecular biology has covered the fields of transcription, translation, and genomic organization, but not DNA replication. One of the clear-cut differences between eubacteria and eucaryotes is the sensitivity of their DNA replicases to the antibiotic aphidicolin. Aphidicolin is an inhibitor of animal α DNA polymerase, α-like DNA polymerase of plants and yeasts, and some animal virus-encoded DNA polymerases (2). Therefore, this drug inhibits the growth of eucaryotic cells by preventing replicative DNA synthesis. In contrast, aphidicolin is inactive on *Escherichia coli* DNA holopolymerase III and T4 phage polymerase (8). We found that DNA replication in toluene-treated *E. coli* cells is also insensitive to this drug (unpublished data). Replication of the bacteriophage ϕ29 is inhibited by aphidicolin but at a dose 100-fold higher than required to inhibit α DNA polymerase activity (10).

As a first step in tackling the problem of DNA replication in archaebacteria, we examined the effect of aphidicolin on growth and macromolecular synthesis in halophilic archaebacteria. These bacteria are extreme halophiles which require from 15 to 25% NaCl for growth (1). We chose these organisms because their only permeability barrier is a cytoplasmic membrane covered with an outer layer of eucaryote-like glycoproteins (5). We observed complete absence of growth in liquid culture in the presence of 20 μg of aphidicolin per ml for all the species of the genus *Halobacterium* tested, i.e., *Halobacterium halobium*, *H. trapanicum*, *H. volcanii*, *H. saccharovorum*, and two haloalkaliphilic bacterial species, SP2 and SP4. Figure 1 shows that *H. halobium* responds to aphidicolin in a dose-dependent manner; 50% inhibition was observed with 3 μg of drug per ml. This dose is in the range of concentrations which inhibit purified eucaryotic α DNA polymerase. Direct comparisons are difficult to make in vivo, because the extent of inhibition of DNA synthesis in eucaryotic cells by aphidicolin is a function of the dCTP pool size (8). The results were reproducible only if fresh solutions of aphidicolin were used.

H. halobium continued to increase in cell mass for several hours after addition of aphidicolin (20 μg/ml) in mid-log phase (Fig. 2) (the generation time under these conditions was 10 h). Therefore, the inhibition by aphidicolin cannot be attributed to a detergent effect as was reported for rifampin by Zillig et al. (12). Microscopic examination showed a dramatic effect of aphidicolin on cell shape: they became elongated as long rigid rods. The number of viable cells stopped increasing immediately after addition of aphidicolin (Fig. 2). Until 40 h after addition of the drug, this number stayed constant and resumption of growth occurred after removal of the drug as is the case with eucaryotic cells. After this step, the number of CFU decreased exponentially.

To determine more precisely the intracellular target of aphidicolin, we studied the effect of this drug on macromolecular synthesis in *H. halobium*. Figure 3 shows that in the first 30 h after addition of aphidicolin, [^{3}H]thymidine incorporation was inhibited much earlier than [^{3}H]uridine incorporation, and aphidicolin did not inhibit protein synthesis. The incorporation of [^{3}H]thymidine was stopped immediately at 20 μg/ml (Fig. 3A), whereas the incorporation of [^{3}H]uridine stopped only after 15 h (Fig. 3B). Extraction of the nucleic acids and their digestion with RNase and DNase showed that [^{3}H]thymidine was exclusively incorporated in DNA. These results indicate that DNA synthesis is the intracellular target of aphidicolin in *H. halobium*. Since the inhibition by aphidicolin was immediately reversible, arrest of DNA synthesis cannot be due to DNA degradation. An appealing hypothesis is that an α-like DNA polymerase is involved in the replication of the *H. halobium* chromosome. Our hope is that aphidicolin will help us to detect archaebacterial DNA replicases and isolate DNA replication mutants in these organisms. Aphidicolin could also facilitate the discrimination between eubacterial and archaebacterial species.

To determine whether aphidicolin sensitivity is a general property of archaebacteria, we tested the effect of this drug on the growth of the thermoacidophilic strain *Sulfolobus acidocaldarius*, which belongs to a different archaebacterial branch than the extreme halophiles (9). We found no effect of the drug to a concentration of 25 μg/ml. Since aphidicolin was still active on *H. halobacterium* after preincubation for several hours at high temperature (75°C) in acidic culture medium of *S. acidocaldarius*, the resistance of this organism may reflect either the absence of an intracellular target for the drug or impermeability to aphidicolin. We have partially purified a thermophilic DNA polymerase from *S. acidocaldarius* and found it to be resistant to aphidicolin. The possibility nevertheless remains that the enzyme is not the *S. acidocaldarius* replicase.

Until now, several eucaryotic-like features have been detected in the transcription and translation apparatuses of archaebacteria (3–5). Our data suggest that archaebacteria and eucaryotic cells also share common characteristics of DNA replication.

* Corresponding author.

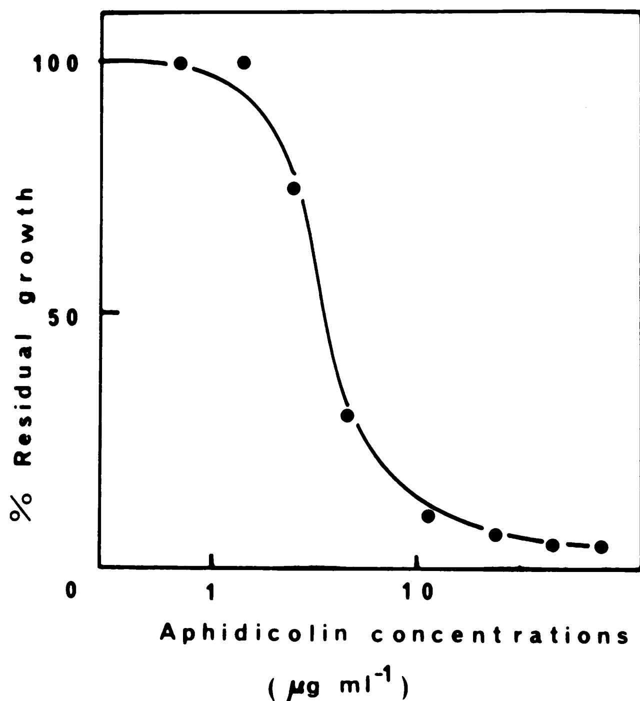

FIG. 1. Inhibition of *H. halobium* growth by aphidicolin. *H. halobium* CCM2090 was grown at 37°C without agitation in classical halophile medium (7). Several test tubes containing 2 ml of medium each and various concentrations of aphidicolin were inoculated with 50 μl of a late-log-phase culture. After 8 days, the tubes were agitated to mix the upper layer of red vacuolated bacteria. The optical density was then measured at 600 nm. In the absence of drug, the optical density varied from 9 to 11 units according to the experiment. Aphidicolin was diluted in distilled water from stock solutions (10 mg/ml) in dimethyl sulfoxide. Dimethyl sulfoxide did not affect *H. halobium* growth up to a final concentration of 1%.

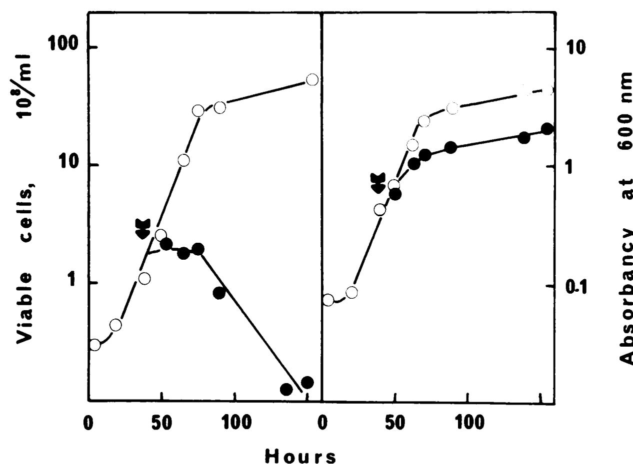

FIG. 2. Kinetics of *H. halobium* growth in the presence of aphidicolin. *H. halobium* CCM2090 (25 ml) was grown as described in the legend to Fig. 1. Aphidicolin was added when the culture reached an optical density at 600 nm of 0.5. Viability counts were determined on samples appropriately diluted into classical halophile medium broth and plated on classical halophilic medium agar plates. The plates were incubated for 10 days at 42°C in a plastic bag with wet cotton wool. Symbols: ○, no aphidicolin; ●, 20 μg of aphidicolin per ml. The arrows show the time of aphidicolin addition.

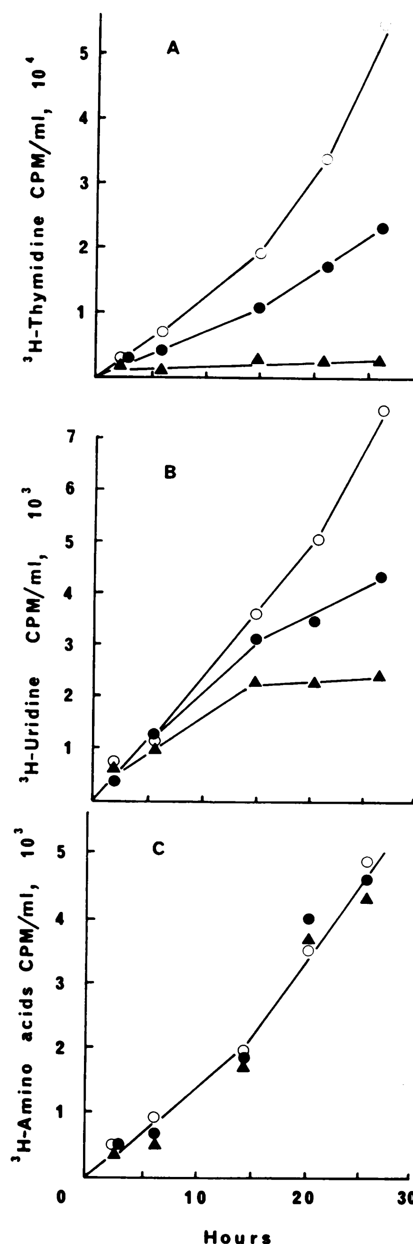

FIG. 3. Effect of aphidicolin on macromolecular synthesis in *H. halobium*. *H. halobium* CCM2090 (250 ml) was grown as described in the legend to Fig. 1. At an optical density of 0.07, three subcultures (75 ml each) received (A) [^{3}H]thymidine (150 μCi, 28 Ci mmol^{-1}) and 1.5 mg of deoxyuridine; (B) [^{3}H]uridine (75 μCi, 30 Ci mmol^{-1}); and (C) ^{3}H-amino acids (leucine, serine, valine, and isoleucine, 100 μCi, 30 Ci mmol^{-1}). At an optical density of 0.2, each subculture was divided into three samples, and aphidicolin was added to two of them (5 and 20 μg/ml). At the time indicated, 0.5 ml of culture was precipitated by the addition of 5% trichloroacetic acid and 0.02 M sodium PP_i. The values for [^{3}H]uridine incorporation into RNA were calculated by taking into account the fact that 35% of the trichloroacetic acid-precipitable radioactivity corresponded to DNA as determined by both RNase digestion and alkaline hydrolysis. Symbols: ○, no aphidicolin; ●, 5 μg of aphidicolin per ml; ▲, 20 μg of aphidicolin per ml.

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