Visualization of Genetic Transcription

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Transcription of structural genes by RNA polymerase to produce messenger RNAs (mRNAs) and the translation of such messengers by polyribosomes to produce proteins are intimately coupled processes in bacterial cells (1). In fact, it is possible to reconstruct coupled transcription and translation systems in vitro from separated bacterial components (2,3). Using techniques developed by Miller and Beatty (4) to prepare the nuclear contents of amphibian oocytes for electron microscopy, we can now directly visualize genetically active bacterial chromosomes. In these studies, we have identified both structural genes and ribosomal RNA (rRNA) genes (5,6).

TECHNIQUE

Bacterial cultures in log phase are rendered osmotically sensitive by a brief treatment with T4 lysozyme in the presence of sucrose at 4°. The protoplasts are then osmotically shocked by rapid dilution into distilled water, and a sample of burst cells is deposited onto a carbon-coated electron microscope grid by low-speed centrifugation. Preparations are stained with phosphotungstic acid (PTA) under conditions that stain net positive groups of proteins (7). Therefore, all the structures observed have protein associated with them.

STRUCTURAL GENES

At low magnification, the extruded contents of a shocked bacterial cell
appear as a network of deoxyribonuclease-digestible fibers with attached ribonuclease-sensitive polyribosomes (Fig. 1). The fibers are stained with PTA and measure approximately 40 Å in diameter, twice the diameter of duplex DNA. Thus the bacterial chromosome appears to be uniformly coated with protein. The protein may become associated with the DNA during isolation, or the DNA may in fact exist as a deoxyribonucleoprotein complex in the cell, as suggested by the experiments of Zubay and Watson (8). In Chapter 3 of this volume, August et al. (9) describes the isolation of several classes of histone-like proteins that could associate with the bacterial chromosome.

Figures 2 and 3 show relatively long regions of the genome exhibiting short-to-long polyribosome gradients. Since the length of genetically active DNA in both figures is sufficient to code for several proteins,* we conclude that these active segments are operons exhibiting intimately coupled transcription and translation. The absence of free polyribosomes on grids or in the supernatant

* Lactose operon (three genes), approximately 1.4 μ; tryptophan operon (five genes), approximately 2.3 μ.