Control of Bacteriophage φX174 Gene Expression by the Transcription Termination Factor ρ in vitro

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Summary. The effect of ρ factor on bacteriophage φX174 gene expression was studied in a cell-free, DNA-dependent, RNA-directed protein synthesizing system (transcription-translation coupled system) using S30 extracts of wild-type and ρ-mutant Escherichia coli cells. It has been found that in the presence of a functionally active endogenous or added ρ factor, only a limited number of the viral genes (A, B, and D) are expressed effectively, whereas the expression of genes, F, G, and H, which encode capsid proteins, is strongly inhibited. Mutational or thermal inactivation of ρ factor results in a considerable activation of capsid protein synthesis and, at the same time, in some depression of the synthesis of gene A, B, and D products. From the results of this study it is suggested that phage φX174 has two classes of genes, late and early, which are separated on the chromosome by a ρ-dependent transcription terminator. We propose that the expression of these classes is regulated by a termination-antitermination mechanism.

Introduction

Bacteriophage φX174 belongs to the group of small isometric coliphages whose chromosome is covalently closed single-stranded DNA (plus-chain) with a molecular weight of about 1.6 × 10⁶ (Denhardt 1977). The primary structure of φX174 DNA is now fully known and the major genes of the phage have been strictly localized (Sanger et al. 1977, 1978) (Fig. 1). After the plus-chain of the DNA has entered the cell, a complementary minus-chain is synthesized, which results in the formation of a double-stranded, covalently closed supercoiled DNA molecule (RFI DNA). The parent molecule of RFI DNA is synthesized by proteins of the host bacterium, and this synthesis appears to separate, as it were, all genes of the phage into two groups: A-J and F-H. Proteins of the first group are mainly involved in cellular processes, whereas those of the second group make up the viral capsid. This arrangement suggests that the expression of the genes is coordinated and is controlled by ρ-dependent termination.

In the middle of the infection cycle, two important regulatory events occur: the replication of RFI DNA and e. coli DNA ceases and single-stranded (SS) viral DNAs begin to form. The products of most phage genes, including genes F, G and H which are the main structural components of the capsid (Burgess 1969), are involved, along with bacterial proteins, in the formation of these SS DNAs. The participation of F, G, and H proteins in this process is accounted for by the fact that synthesis of SS DNA is closely coupled with the morphogenesis of the phage particle and can take place only during capsid formation (Hayashi 1978). The development of bacteriophage φX174 is therefore a strictly ordered chain of events. However, the mechanisms underlying these events are still unclear. Studies, both in vivo and in vitro, on the transcription of φX174 genes have revealed, on the φX174 chromosome, three major promoters for the e. coli RNA polymerase: P A, P b, and P d located before genes A, B, and D, respectively. Moreover, several additional sites of RNA synthesis initiation have been discovered (Axelrod 1976; Hayashi et al. 1976; Kapitza et al. 1976, 1979; Smith and Sinsheimer 1976). At the same time, ρ-dependent (T ρ ) and ρ-independent terminators have been identified and localized approximately (see Fig. 1), although their significance in the life cycle of the phage remains obscure. Nevertheless, one of the ρ-dependent terminators, which is located at the beginning of gene F, appears to separate, as it were, all genes of the phage into two groups: A-J and F-H. Proteins of the first group are mainly involved in cellular processes, whereas those of the second group make up the viral capsid. This arrangement suggests that the expression of the genes is coordinated and is controlled by ρ-dependent termination.

Accordingly, we have studied the effect of ρ factor on the formation of φX174 proteins in a cell-free, DNA-dependent, RNA-directed protein synthesizing system (transcription-translation coupled system). We have found that synthesis of φX174 capsid proteins does not occur in the presence of a functionally active ρ factor. Inactivation of ρ factor results in resumed synthesis of these proteins. A preliminary report has been published elsewhere (Patrushev et al. 1980).

Materials and Methods

Bacterial and Phage strains. E. coli W3100 (wild type) and psu4 (ρ-mutant) were provided by Ch. Yanofsky (Korn and Yanofsky 1976).
Expression of Genes F, G, and H in S30 Extracts of Wild-Type Cells

In view of the instability of the termination factor $\rho$ (Roberts 1969), we modified somewhat the conditions of preparation of S30 extracts by substituting a milder preincubation (30°C for 30 min) for a longer-lasting incubation of extracts at 37°C, which is used to deplete endogenous templates (Zubay et al. 1970). As can be seen from Fig. 2 (lane 1), these preincubation conditions resulted in the complete disappearance of DNA-independent protein synthesis. When S30 extracts of wild-type cells obtained under the milder conditions were used, only a limited number of phage proteins, mostly those with low molecular weights, were formed in a RFI DNA-dependent, cell-free system. On the other hand, when S30 extracts were preincubated at 37°C for 1 h, the spectrum of proteins synthesized was rather different, higher molecular weight proteins having appeared (Fig. 2, lane 6). The same effect was observed when S30 extracts were exposed to 42°C for 10 min (Fig. 2, lane 3). When preincubation was extended to 20 min at the same temperature, the relative amount of high-molecular weight proteins was higher (Fig. 2, lane 4); however, protein synthesis was generally weakened in this case, probably because of thermal inactivation of the transcription and translation systems in the cell-extracts.

The principal products of $\phi$X174 genes synthesized in vitro were identified as templates $\phi$X174 RFI DNA preparations containing amber mutations in particular genes (Fig. 3). It can be seen that A, B, and D proteins were synthesized in extracts of wild-type cells and that the synthesis of capsid proteins F, G, and H was greatly reduced in these extracts (Fig. 3, lanes 1–3). Exposure of the extracts to 42°C for 10 min had a stimulatory effect on the synthesis of capsid proteins (Fig. 3, lanes 4–6). Con-