# ΦX-174 COAT PROTEIN MUTANTS AFFECTING DNA SYNTHESIS

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Amber mutants of phage ΦX-174 differing from wild type phage in sensitivity to heat inactivation, and therefore designated coat protein mutants, show unusual patterns of DNA synthesis in their restrictive hosts. These mutants fall into the single complementation group: A. In the restrictive host normal amounts of replicative form DNA (RF-DNA) are produced, but the amount of single strand phage DNA (Φ-DNA) is reduced, and a third form of DNA appears which is single stranded, non-infectious, and phage specific (A-DNA).

Mutants N-1 and H-190, produced with nitrous acid and hydroxyl amine respectively, are able to grow on Escherichia coli strain CR 63.1 ($sus^+$) but not on strain C ($sus^-$). The cells were grown at 37°C in SCXD medium (Siegel & Hayashi, 1967) and infection was synchronized by NaCN treatment modeled after Denhardt and Sinsheimer (1965). Infected cells were diluted into 37°C SCXD containing $^3$H-Thymidine (1.1 μg/ml, spec. act. = 2 x $10^{11}$ cpm/mM), harvested by filtration at the times indicated, and the nucleic acids extracted (Siegel & Hayashi, 1967).

Components of the DNA extract were resolved by zone sedimentation at 18°C on gradients of 5%-20% sucrose in 0.005 M Na-EDTA buffer, pH 10.0. The 21 s and 16 s allomorphs of RF-DNA and single strand phage DNA are separable (Figure 1).

We have examined about 100 mutants of ΦX-174 which fall into seven different complementation groups: A through G. These groups have been assigned functions by complementation studies with S-13 mutants of known

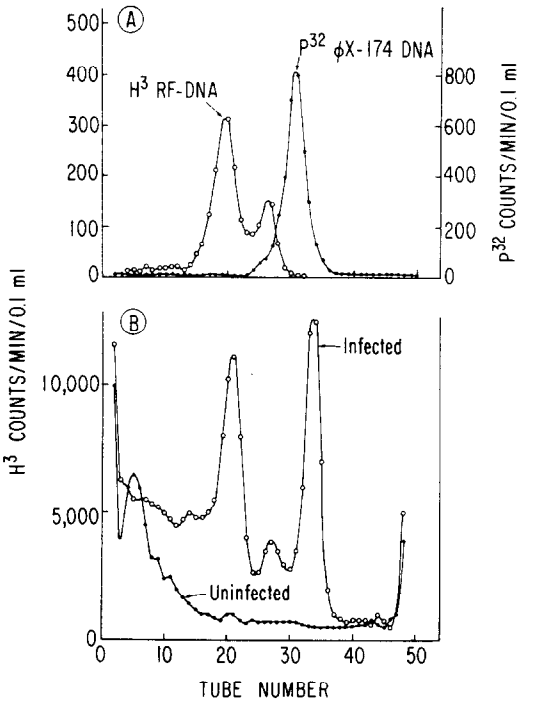


Figure 1: (A) Reconstruction experiment: Unlabeled E. coli DNA from $10^8$ cells, purified $^{32}P$ labeled ΦX-174 DNA, and $^3H$-labeled RF-DNA were centrifuged in an SW-50 rotor at 50,000 RPM for 120 minutes at 18°C. Fractions were collected on filter papers, washed with 5% TCA, dried and counted. $^3H$-RF-DNA —o—o—o—, $^{32}P$ ΦX-174 DNA —·—·—·—. (B) Nucleic acids from E. coli C (sus⁻): infected with wild type ΦX-174 and extracted 20 minutes after infection —o—o—o—: and from uninfected cells —·—·—·—. Sedimentation as in A.

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function (Tessman, I., et al., 1967), by heat inactivation studies of mature phage (Jeng & Hayashi, 1968), and by examination of the DNA produced in infected C (sus⁻) cells (Siegel, Hayashi & Hayashi, 1968). The mutants described herein fall into a single complementation group: group A (Tessman's group IIIb). These mutants differ in heat sensitivity from wild type ΦX-174 (Jeng & Hayashi, 1968; Tessman & Tessman, 1966).

The sedimentation patterns of nucleic acids from E. coli C (sus⁻) uninfected and infected with wild type ΦX-174 are shown in Figure 1-B. Group A mutants grown on E. coli C (sus⁻) for 20 minutes give the nucleic acid components shown in Figure 2-A; the extra DNA component at approximately

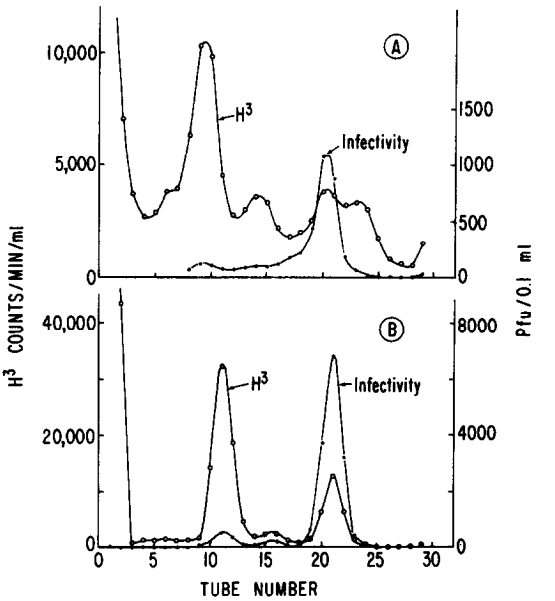


Figure 2: Zone sedimentation in SW-25.1 rotor at 25,000 RPM for 14 hours at 18°C. (A) Nucleic acids extracted from E. coli C ($sus^-$) infected with A mutant (N-1). (B) Nucleic acids from CR 63.1 ($sus^+$) infected with A mutant (N-1). $^3$H-DNA –o–o–o–; infectivity on spheroplasts –.–.–.–.

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9 s (A-DNA) is not found in CR 63.1 infected with A mutants (Figure 2-B). The infectivity of the various DNA forms assayed on spheroplasts of E. coli HF 4714 ($sus^+$) (Guthrie & Sinsheimer, 1963) is shown in Figure 2 A & B. The infectivity represents more than a fifteen fold increase over uninjected phage DNA present at 6 minutes, prior to the onset of phage DNA synthesis.

In an effort to ascertain the nature of the A-DNA we carried out hybridization experiments. DNA extracted from N-1 infected E. coli C ($sus^-$) was resolved on sucrose gradients and individual fractions were hybridized against E. coli DNA, RF-DNA, and phage DNA (Φ-DNA) as described by Warnaar and Cohen (1966) (Figure 3-A). Further, A-DNA was compared to DNA extracted from 70 s ΦX-174 aberrant phage particles (Figure 3-B). The DNA from these particles has a molecular weight of $2.6 \times 10^5$ Daltons and is linear and single stranded (Eigner, et al, 1963). From the hybridization and sedimentation studies it can be seen that the A-DNA component is single stranded, contains nucleotide

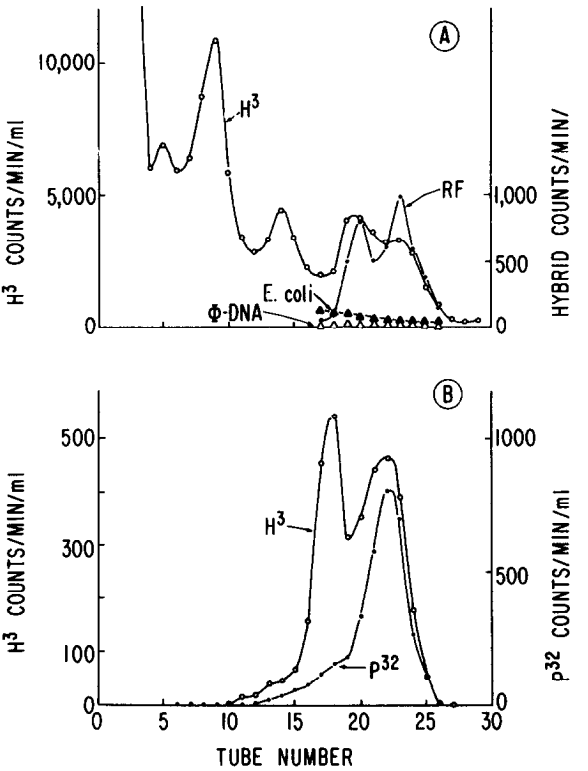


Figure 3: (A) Sedimentation as in figure 2. $^{3}H$ nucleic acids extracted from E. coli C (sus$^-$) infected with N-1 —o—o—o—. DNA in fractions indicated was hybridized vs. RF-DNA —·—·—·—, E. coli C (sus$^-$) DNA —▲—▲—▲—, and phage ΦX-174 DNA —Δ—Δ—Δ—. (B) DNA purified from Φ-DNA and A-DNA region of 3-A was centrifuged for 20 hours as per figure 2. $^{32}P$ DNA extracted from purified 70 s phage particles was added as a marker. $^{3}H$ DNA —o—o—o—, $^{32}P$ phage DNA ·—·—·—·.

sequences homologous with the original phage DNA, is probably linear and has a molecular weight similar to DNA from 70 s phage particles.

Growth of A mutants on two other sus$^-$ strains of E. coli, T$^-$H$^-$U$^-$ (Stern et al, 1964) and HF 4704 (obtained from R. L. Sinsheimer), gave similar results to mutants grown in C (sus$^-$).

Phage cistrons specifying the structure of proteins having enzymatic, structural, or regulatory functions are known. There have been prior reports of phage cistrons which affect more than one function in the phage life cycle. Zinder and Lyons (1968) reported that the coat protein of f-2, which

has a known regulatory function (Sugiyama & Nakada, 1967), is necessary for lysis of the infected cell. The A cistron in ΦX-174 apparently specifies a coat protein and plays some role in single strand DNA synthesis.

It is not yet known if the new form of DNA is a precursor or a product of the phage DNA. The former possibility is more interesting but the latter could arise if, for example, altered coat proteins prevented completion of phage particles and left incompletely packaged phage DNA exposed to the ravages of the cellular environment. The possibility that the A mutants are polar and exert their effect on DNA synthesis by decreasing the production of a protein distal to A is unlikely since several different A mutants show this aberrant form of DNA, and because both mutants examined complement well with mutants in all other cistrons.

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REFERENCES

Denhardt, D. and Sinsheimer, R. L., J. Mol. Biol. 12, 641 (1965).
Eigner, J., Stouthamer, A., van der Sluys, I., and Cohen, J., J. Mol Biol. 6, 61 (1963).
Guthrie, G. D. and Sinsheimer, R. L., Biochim. Biophys. Acta 72, 290 (1963)
Jeng, Y. and Hayashi, M., 1968, in preparation.
Siegel, J. and Hayashi, M., J. Mol. Biol. 27, 443 (1967).
Siegel, J., Hayashi, M. N., and Hayashi, M., 1968, in preparation.
Stern, J., Sekiguchi, M., Barner, H., and Cohen, S., J. Mol. Biol. 8, 629 (1964).
Sugiyama, T. and Nakada, D., Proc. Natl. Acad. Sci. 57, 1744 (1967).
Tessman, I., Ishiwa, H., Kumar, S., and Baker, R., Science 156, 824 (1967).
Tessman, I. and Tessman E., Proc. Natl. Acad. Sci. 55, 1459 (1966).
Warnaar, S. and Cohen, J., Biochem. and Biophys. Res. Comm. 24, 554 (1966).
Zinder, N. and Lyons, L. B., Science 159, 84 (1968).