*THE ROLE OF THE N GENE OF PHAGE λ IN THE SYNTHESIS OF TWO PHAGE-SPECIFIED PROTEINS**

By C. M. Radding† and H. Echols

DEPARTMENT OF HUMAN GENETICS, UNIVERSITY OF MICHIGAN, ANN ARBOR, AND DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN, MADISON

Communicated by James V. Neel, March 29, 1968

The biosynthesis of bacterial viruses, starting with the nucleic acid and ending with the extracellular virus particles, comprises a number of steps subject to regulation by viral genes.[1, 2] At least two stages, early and late, can be distinguished in the vegetative growth of the temperate phage λ. These stages can be seen in the kinetics of synthesis of phage DNA, messenger RNA (mRNA), and proteins,[3, 4] as well as in the genetic control of phage development.[1, 5, 6] Two λ genes appear to play a critical role in this ordered development. Mutations in gene *Q* cause multiple late defects,[7] including reduced synthesis of mRNA[8] and late proteins.[7] Mutations in gene *N* lead to multiple defects in both early and late functions. The early defects include DNA replication,[3, 6, 9] early messenger synthesis,[8] and the synthesis of two λ proteins, exonuclease[9–11] and a recently discovered antigenic protein called β.[11] The other known early mutants of λ that are defective in the replication of DNA do not alter the synthesis of exonuclease[9–11] or β protein.[11] Thus mutations in gene *N* affect the largest number of early functions, and *N* would appear to be responsible for some critical event in the initiation of phage development. As a readily measured parameter of the action of *N*, the synthesis of exonuclease and β protein is useful for studying the mechanism by which gene *N* works.

Three possible explanations existed for the role of gene *N* in the synthesis of exonuclease and β protein: (1) *N* might be the structural gene for either or both proteins; (2) *N* might not be the structural gene for either protein, but the *N* mutations studied might have a polar effect on synthesis by the nearby exonuclease and β genes; and (3) *N* might be the structural gene for a protein that must act as a prerequisite for the synthesis of exonuclease and β protein. This report describes experiments that support the last hypothesis and further characterize the mechanism of action of gene *N*.

Materials and Methods.—(a) *Phage and bacterial strains:* The strains of *E. coli* used and their relevant genetic characteristics were the following: Hfr H su^-, the parental su^- strain; CA161 su_{II}^+ and CA 169 su_c^+, nearly isogenic su^+ derivatives carrying, respectively, the *amber* suppressor su_{II}^+ and the *ochre* suppressor su_c^+ (from J. R. Beckwith);[12] C600 su_{II}^+ and W3350 su^-, standard permissive and nonpermissive hosts, respectively, for the Campbell λ *sus* nonsense mutants.[5] The phage strains used were the following: λ *sus* mutants N_{53} and J_{27};[5] and λ Nts_8, λ Nts_9, and λ Nts_{10}[13] (all from A. Campbell).

(b) *Assays of exonuclease, β protein, and tail antigen:* Exonuclease, β protein, and tail antigen of phage λ were assayed in extracts from lysogenic cultures treated with mitomycin C to release repression. The preparation of extracts, assay of exonuclease activity, and immunological detection of β protein were carried out as described previously.[11, 14] Tail antigen was assayed as described by Dove,[7] except that the virulent phage λ_v,[15] instead of λh, was used as the test phage for tail antigen, and the extracts were dialyzed before assay.[16]

TABLE 1. *Ochre suppression of N_{53} and J_{27}.*

Prophage	Suppressor	Exonuclease	Tail antigen	Phage/cell
λ^+	su^-	4.9	100	48
J_{27}	su^-	3.3	<0.3	8×10^{-5}
N_{53}	su^-	$\leqslant 0.04$	—	1×10^{-4}
λ^+	su_c^+	4.9	100	44
J_{27}	su_c^+	6.8	~0.5	1
N_{53}	su_c^+	5.4	—	11

Either Hfr H su^- or CA169 su_c^+ lysogens carrying the above prophages were grown in T-broth (1 % tryptone, 0.5 % NaCl) at 33°C to a density of approximately 2×10^8 cells/ml. The temperature was then raised to 37°C, and mitomycin C was added at a final concentration of 2 μg/ml. Aliquots for assay of exonuclease, β protein, and tail antigen were taken at 70 min, and the phage yield was assayed after treatment with $CHCl_3$ at 150 min. Phage titers were determined using C600 su_{II}^+ as the host. The assays for exonuclease[14] and tail antigen[7, 16] are described elsewhere. The unit of exonuclease activity is defined in terms of the difference between (H^3)-DNA degradation in the presence and in the absence of antiexonuclease serum.[11] The exonuclease specific activities are expressed as units/mg protein. The values for tail antigen are expressed as per cent of the λ^+ control; the actual data in phage equivalents/ml/mg protein were 4×10^{11} for su^- (λ^+) and 5×10^{11} for su_c^+ (λ^+).

Results.—(a) *Ochre suppression of a nonsense mutation in the N gene:* An opportunity to choose among the hypotheses proposed above (see introduction) is provided by studying the quantitative relationship between the synthesis of exonuclease and β protein, and the synthesis of the product of the N gene. Variation in the amount of the N product can be achieved experimentally by inefficient *ochre* suppression of an *amber* or *ochre* nonsense mutation in gene N. *Ochre* suppression provides for completion of the otherwise interrupted polypeptide product of the mutated gene,[12] but completed proteins are formed at a much lower rate than in the absence of a mutation in the relevant structural gene.[17–19] If N were the structural gene for either or both exonuclease and β, then very little of one or both of these proteins should be made under conditions of *ochre* suppression of an N mutation. Similarly, if the N nonsense mutation affects exonuclease and β production by a polar mechanism, then polarity should be eliminated only to the extent that the synthesis of N product is restored,[20, 21] and little exonuclease and β protein should be made. On the other hand, if the synthesis of exonuclease and β protein requires the action of a different protein made by gene N, a protein that might be present in excess, then the small amount of N protein resulting from inefficient *ochre* suppression might suffice to effect normal synthesis of exonuclease and β protein.

Suppression of the *amber* mutation N_{53} by the *ochre* suppressor su_c^+ (ref. 12) was studied. As a control for inefficient suppression by su_c^+, we also studied suppression of the *amber* mutation J_{27}, which is in a gene controlling the structure of an antigenic tail protein.[7] Table 1 shows the effect of *ochre* suppression on the production of exonuclease, tail antigen, and viable phage. In the absence of suppression, λJ_{27} made no detectable tail antigen, and λN_{53} made no significant exonuclease activity, as expected from previous results.[7, 10, 11] In the presence of su_c^+, λN_{53} made as much exonuclease as the wild-type controls and gave a 25 per cent yield of phage; λJ_{27} produced very little tail antigen and a much lower yield of phage than λ^+ or λN_{53}.

In addition, the synthesis of β protein was examined by immunodiffusion assay (Fig. 1). As described before, a *sus N* mutant in a su^- host made little or no de-

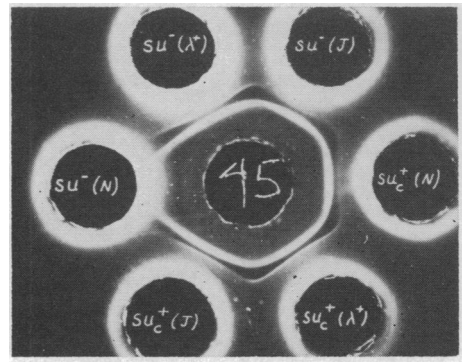

FIG. 1.—Synthesis of β protein after *ochre* suppression of N_{53}. The β-protein immunodiffusion assay shown above was carried out as described previously.[11] The extracts in the outer wells were the same as those assayed for exonuclease (Table 1). The center well contained antiserum to exonuclease and β protein. The exonuclease precipitin band is farthest from the center well; the β-protein precipitin band is closest to the center well.

tectable β protein;[11] by contrast, *ochre*-suppressed λN_{53} made an amount of β protein that was comparable to that of the wild-type controls, as judged from the density and position of the β-precipitin band. Although the β protein in this experiment was not assayed quantitatively, the result is clearly distinguishable from that expected from *ochre* suppression of a mutant in the β structural gene. Even if *ochre* suppression of a mutant in a structural gene had restored polypeptide synthesis with an efficiency as high as 20 or 30 per cent, the resulting β-precipitin reaction would have been discernibly weaker than that of the wild-type controls. (Cf. ref. 11.)

The results with λJ_{27} show that su_2^+ *ochre* suppression of a mutation in a structural gene for tail antigen produces very little tail antigen, as expected from the work of others on su_c^+ suppression of mutants for T4 head protein[17] and β-galactosidase.[18] Su_{II}^+ *amber* suppression of λJ_{27} led to the production of 14 per cent of the wild-type level of tail antigen, showing that λJ_{27} was suppressible. Thus the strain carrying su_c^+ was inefficient in restoring the synthesis of a protein determined by a λ gene bearing an *amber* mutation. Inefficiency of *ochre* suppression of *amber* mutations is further supported by the finding that no λ *amber* mutant (of 22 tested) in genes specifying head and tail proteins showed an efficiency of plaque formation in the case of *ochre* suppression as high as that found in the case of *amber* suppression.[22] We would expect that the head and tail proteins would have to be present in nearly normal amounts for efficient phage production.

Since very little of the product of gene N is therefore likely to have been formed by su_c^+ suppression of the *amber* mutation N_{53}, the normal synthesis of exonuclease is readily understandable only if N is *not* the structural gene for exonuclease and if the product of N is required for the synthesis of exonuclease. Similarly, synthesis of β protein by the *ochre*-suppressed N_{53} was comparable to synthesis by the wild-type controls and clearly greater than that expected from *ochre* suppression of a structural gene for β. It is likely, therefore, that N is not the structural gene for β protein either and that the product of N is required to start β synthesis.

(b) *Thermosensitive synthesis of exonuclease:* Nonsense mutations exert a polar effect, diminishing the synthesis of the product of other genes of the same operon distal to the operator site.[20, 21, 23] No polar effect of missense mutations has been found.[20, 23] Therefore, given that N is not the structural gene for exonu-

TABLE 2. *Thermosensitive synthesis of exonuclease.*

Prophage	Exonuclease (U/mg) 30°C	37°C	43°C	Infective Centers (% of Total Cells) 30°C	37°C	43°C
λ^+	2.0	13.0	14.0	41	86	100
λts_9	0.06	2.2	0.27	1.0	10	<0.01

W3350 *su*⁻ lysogens carrying the above prophages were grown to 1–2 × 10^8 cells/ml in T-broth at the indicated temperatures and induced with mitomycin C at 6 μg/ml. Aliquots for exonuclease were taken at 60 min. The units of exonuclease are the same as in Table 1. The fraction of cells producing phage after mitomycin treatment was determined by plating the induced cells on W3350 *su*⁻ in a standard assay for infective centers. In this assay, plates were incubated at the same temperature as that used for growth; the assay at 43°C employed plates warmed as described by Brown and Arber.[13]

clease,[24–26] a demonstration that missense mutations in gene *N* can prevent exonuclease synthesis would constitute a strong argument against a polar mechanism for the reduced synthesis of exonuclease by *N* mutants. Mutations that have thermosensitive expression are very likely to be missense mutations leading to the production of a thermosensitive product. The exonuclease activity of three thermosensitive mutants in *N* has been examined. These are mutants that map in the *N* cistron[13, 27, 28] and that form plaques at 30°C (or 37°), but not at 42°. In an initial experiment, the exonuclease activity was measured in lysogens induced and held at 37°. The exonuclease activity of Nts_8, Nts_9, and Nts_{10}, respectively, was 0.7, 0.7, and 0.5 per cent of that of a wild-type control. One of these, ts_9, was studied in more detail (Table 2). Optimal production of phage and exonuclease activity was observed for the mutant at 37°; at 43°, the yield of ineffective centers and enzyme activity was further reduced. By contrast, the yield of infective centers and enzyme by the wild-type control was the same at 43° as at 37°. These results show that the production of exonuclease activity as well as phage is rendered thermosensitive by these mutations in gene *N*. Thus, in agreement with the experiments described above, polarity is an unlikely explanation for the failure of *N* mutants to synthesize exonuclease normally. On the contrary, these observations, together with the demonstration that *N* is not the structural gene for exonuclease, provide independent evidence that the product of *N* is required for the synthesis of exonuclease.

Discussion.—Other experiments have shown that the structural genes for exonuclease and β protein are distinct from gene *N* and map to the left of it.[24–26] The experiments reported here agree with the conclusion that *N* is not the structural gene for either protein and, in addition, characterize the functional relationship between *N* and the synthesis of exonuclease and β protein. The product of gene *N* is apparently sufficient in reduced amounts to permit the synthesis of normal amounts of exonuclease and β protein. The apparent excess of *N* product present in wild-type controls probably reflects a mechanism by which a few molecules of *N* product suffice for the synthesis of many molecules of exonuclease and β protein. For example, the product of *N* might be responsible for some initiating event, after which the synthesis of exonuclease and β protein, among other syntheses, proceeds without further need for the initiator; or the product of *N* might act continuously but catalytically to support or stimulate the synthesis of some early proteins. However, the data do not exclude the possibility that each molecule of *N* product effects the synthesis of one molecule each of exonu-

clease and β protein since we have no measurement of the molar ratio of N product to exonuclease and β protein. Further experiments are required to distinguish between such alternatives, but in any case, the product of N is required for the synthesis of exonuclease and β to begin. Other work has shown that the cessation of exonuclease synthesis, and possibly also the rate of synthesis per cell, is under the control of another phage gene or genes.[29, 37]

Others have suggested that the essential role of gene N in vegetative growth may be to induce the expression of the other essential early genes, O and P,[30, 31] which map to the right of gene N.[5] Recently, however, it has been shown that, to some extent at least, genes O and P can function in the absence of N[32] and, at present, there is no critical evidence on the relationship of gene N to the normal expression of the essential early genes located to the right of N. Mutants in N are defective in the synthesis of "early" messenger, which has led to the suggestion that N is required for "high-level" or efficient transcription of early genes.[8] However, it is not possible to specify how the action of N governs early-messenger synthesis. The product of N might act directly in transcription (e.g., as an RNA polymerase), or indirectly through some alteration of the DNA that is also required for normal replication. Since the genes for exonuclease and β protein are not essential for vegetative growth,[26] the role of N in vegetative development cannot be explained by the control exerted by N on the synthesis of these two proteins alone. Thus the apparent inducing effect of the product of N on the synthesis of exonuclease and β protein must be an additional, direct or indirect, result of the action of N. The control exerted by N appears to be positive, as contrasted with the negative nature of repression of the *lac* operon.[33] The relationship of the positive N control to other examples of "positive control"[34–36] should be revealed when the mechanism of action of the product of N is discovered.

Summary.—We have examined the functional relationship between gene N of phage λ and the synthesis of two early λ proteins, the exonuclease and the β protein, by studying inefficient *ochre* suppression of an *amber* mutant in N, and by studying thermosensitive mutants in N. The data are interpreted to mean that the product of gene N acts to initiate and/or support the synthesis of exonuclease and β protein. The amount of N product present is in excess of what is needed to achieve normal synthesis of exonuclease and β protein.

We wish to thank J. R. Beckwith, A. Campbell, and W. Arber for bacterial strains and phage stocks, and R. McMacken for carrying out the tail antigen assay.

* This work was supported by U.S. Public Health Service grants GM 09252 and GM 08407.

† Present address: Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

[1] Jacob, F., C. R. Fuerst, and E. L. Wollman, *Ann. Inst. Pasteur*, **93**, 724 (1957).

[2] Epstein, R. H., A Bolle, C. M. Steinberg, E. Kellenberger, E. Boy De La Tour, R. Chevalley, R. S. Edgar, M. Susman, G. H. Denhardt, and A. Lielausis, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 375.

[3] Joyner, A., L. N. Isaacs, H. Echols, and W. S. Sly, *J. Mol. Biol.*, **19**, 174 (1966).

[4] Protass, J. J., and D. Korn, *J. Biol. Chem.*, **241**, 4175 (1966).

[5] Campbell, A., *Virology*, **14**, 22 (1961).

[6] Brooks, K., *Virology*, **26**, 489 (1965).

[7] Dove, W. F., *J. Mol. Biol.*, **19**, 187 (1966).

[8] Skalka, A., B. Butler, and H. Echols, these PROCEEDINGS, **58**, 576 (1967).
[9] Eisen, H., C. R. Fuerst, L. Siminovitch, R. Thomas, L. Lambert, L. Pereira da Silva, and F. Jacob, *Virology*, **30**, 224 (1966).
[10] Radding, C. M., *Biochem. Biophys. Res. Commun.*, **15**, 8 (1964).
[11] Radding, C. M., and D. C. Shreffler, *J. Mol. Biol.*, **18**, 251 (1966).
[12] Brenner, S., and J. R. Beckwith, *J. Mol. Biol.*, **13**, 629 (1965).
[13] Brown, A., and W. Arber, *Virology*, **24**, 237 (1964).
[14] Radding, C. M., *J. Mol. Biol.*, **18**, 235 (1966).
[15] Jacob, F., and E. Wollman, *Ann. Inst. Pasteur*, **87**, 653 (1954).
[16] McMacken, R., B. Butler, A. Joyner, and H. Echols, in preparation.
[17] Stretton, A. O. W., S. Kaplan, and S. Brenner, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 31 (1966), p. 173.
[18] Beckwith, J., *Biochim. Biophys. Acta*, **76**, 162 (1963).
[19] Gallucci, E., and A. Garen, *J. Mol. Biol.*, **15**, 193 (1966).
[20] Yanofsky, C., and J. Ito, *J. Mol. Biol.*, **21**, 313 (1966).
[21] Newton, W. A., J. R. Beckwith, D. Zipser, and S. Brenner, *J. Mol. Biol.*, **14**, 290 (1965).
[22] Adhya, S., personal communication.
[23] Martin, R. G., D. F. Silbert, D. W. E. Smith, and H. J. Whitfield, *J. Mol. Biol.*, **21**, 357 (1966).
[24] Radding, C. M., J. Szpirer, and R. Thomas, these PROCEEDINGS, **57**, 277 (1967).
[25] Szpirer, J., R. Thomas, and C. M. Radding, in preparation.
[26] Manly, K., E. R. Signer, and C. M. Radding, in preparation.
[27] Naha, P. M., and A. Campbell, *Bacteriol. Proc.*, **65**, 103 (1965).
[28] Brachet, P., personal communication.
[29] Radding, C. M., these PROCEEDINGS, **52**, 965 (1964).
[30] Thomas, R., *J. Mol. Biol.*, **22**, 79 (1966).
[31] Protass, J. J., and D. Korn, these PROCEEDINGS, **55**, 1089 (1966).
[32] Eisen, H., L. Pereira da Silva, and F. Jacob, personal communication.
[33] Jacob, F., and J. Monod, *J. Mol. Biol.*, **3**, 318 (1961).
[34] Garen, A., and H. Echols, these PROCEEDINGS, **48**, 1398 (1962).
[35] Douglas, H. C., and D. C. Hawthorne, *Genetics*, **54**, 911 (1966).
[36] Sheppard, D., and E. Englesberg, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 31 (1966), p. 345.
[37] Radding, C. M., unpublished observations.