Activation and Characterization of the Reovirus Transcriptase: Genetic Analysis

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We studied the ability of chymotrypsin to activate the transcriptases of the three serotypes of reovirus. When we used conditions that reproducibly caused the activation of type 3 transcriptase by chymotrypsin alone, type 2 transcriptase was sometimes activated, and type 1 transcriptase was never activated. Using intertypic recombinants containing various combinations of genome segments from reovirus types 3 and 1, we showed that the M2 segment determined this difference. Biochemical experiments indicated that the digestion of reovirus type 1 by chymotrypsin was blocked at an intermediate stage in uncoating. We found conditions which reproducibly activated the transcriptases of all three serotypes. This allowed us to compare the biochemical properties of the three transcriptases. Although the monovalent cation preferences, divalent cation preferences and optima, and temperature optima of type 1, 2, and 3 transcriptases were indistinguishable, the pH activity curves were reproducibly different. The largest difference was between type 2 and 3 transcriptases; the pH optimum of type 2 transcriptase was lower than the pH optimum of type 3 transcriptase. Using intertypic recombinants containing various combinations of genome segments from reovirus types 2 and 3, we demonstrated that the L1 segment specified this difference.

Purified reovirions contain an RNA-dependent RNA polymerase (transcriptase) (3, 13). Although it has been shown clearly that the transcriptase activity resides in the viral core, it is not known which of the viral polypeptides is responsible for this activity. The following approaches have been tried unsuccessfully in attempts to identify the viral component responsible for the transcriptase: (i) efforts to solubilize the transcriptase from virion cores have not been successful, possibly because only strongly denaturing and inactivating agents are capable of dislodging any core components (18); (ii) although there are temperature-sensitive mutants that contain defects in each of the 10 reovirus double-stranded RNA genome segments, none of the 10 mutant groups displays a temperature-sensitive transcriptase activity (4; unpublished data); (iii) pyridoxal phosphate reversibly inhibits transcriptase activity in intact reovirus cores by binding to polypeptides within the core, and this inhibition is not competitive with four ribonucleoside triphosphates, indicating that pyridoxal phosphate does not act at the site of the transcriptase (9).

There are three mammalian reovirus serotypes, which are designated types 1, 2, and 3. These three serotypes differ in a number of biological and biochemical properties. A large

number of intertypic recombinants have been isolated (10-12), and these recombinants have allowed us to identify the viral double-stranded RNA segment(s) that is responsible for a number of these properties. In this study we compared the transcriptase activities of reovirus serotypes 1, 2, and 3. We identified differences in the conditions required for the activation of the transcriptases of the three serotypes, as well as differences in the characteristics of the transcriptase activities. Using the intertypic recombinants, we were able to identify the viral genes responsible for these differences.

MATERIALS AND METHODS

Virus and cells. Mouse L cells were maintained in suspension cultures as previously described (11). Reovirus serotypes 1 (strain Lang), 2 (strain Jones), and 3 (strain Dearing) have been described and used in this laboratory previously (11).

Virus purification. A total of 2.5×10^8 L cells in a suspension culture were centrifuged and then suspended in Puck medium containing 1% fetal calf serum and 1% 2 M $MgCl_2$ at a density of 10^7 cells per ml. A second-passage reovirus stock was added at a multiplicity of 5 PFU/cell and allowed to adsorb for 30 min at 23°C. The infected cells were diluted to a concentration of 5×10^5 cells per ml with Joklik modified Eagle medium containing 1% fetal calf serum and 4% VSP newborn calf serum (Biocell Laboratories, Carson,

Calif.) and then incubated in suspension at 32°C until the viable cell count decreased to 2.5×10^5 cells per ml (usually 72 h). The infected cells were centrifuged, the supernatant was discarded, and the pellet was suspended in 10 ml of homogenization buffer (0.01 M Tris, pH 7.4, 0.25 M NaCl, 0.01 M β -mercaptoethanol). The cells were disrupted by 10 s of sonication at full power with a Braunsonic model 1510 sonicator. Sodium deoxycholate was added to a final concentration of 0.1%, and the preparation was incubated at 4°C for 30 min. An equal volume of trichlorotrifluoroethane (Freon) (Fisher Chemical Co., Pittsburgh, Pa.) was added, and the mixture was emulsified by sonication for 30 s at full power. The emulsion was centrifuged for 5 min at $10,000 \times g$, and the resolved aqueous phase was removed and extracted with 1 volume of Freon. The resulting aqueous phase was layered directly onto a preformed CsCl gradient (density, 1.2 to 1.4 g/ml) in an SW41 rotor tube and centrifuged at $130,000 \times g$ for 2 h. The virus band was collected, dialyzed extensively against 150 mM NaCl–7.5 mM $MgCl_2$ –5.0 mM Tris (pH 7.4), and stored at 4°C. Virus concentration was determined by absorbance at 260 nm (1 U of optical density at 260 nm was equivalent to 2.1×10^{12} particles per ml).

Digestion and transcriptase reactions. Digestion and transcriptase reactions were performed by a modification of the method of Joklik (6). Each digestion reaction mixture contained $1 \times$ SSC (0.15 M NaCl plus 0.015 M sodium citrate), 10^{12} reovirus particles, and 100 μ g of chymotrypsin per ml in a total volume of 100 μ l. The reaction mixture was incubated for 30 min at the specified temperature, after which the reaction was stopped by adding 1 μ l of 0.1 M phenylmethylsulfonyl fluoride and 100 μ l of $1 \times$ SSC at 4°C. Transcriptase reactions were performed in 250- μ l volumes containing 100 mM Tris (pH 8.0) (unless otherwise specified), 10 mM $MgCl_2$, 3.3 mM phosphoenolpyruvate, 100 μ g of pyruvate kinase per ml, 2.0 mM ATP, 2 mM GTP, 2 mM CTP, 2 mM UTP, 250 μ g of bentonite per ml, 2.0 μ Ci of [α - 32 P]UTP, and 100.0 μ l of digestion mixture. [α - 32 P]UTP was obtained from Amersham Corp., Arlington Heights, Ill., phenylmethylsulfonyl fluoride, phosphoenolpyruvate, pyruvate kinase, and nucleotide triphosphates were obtained from Sigma Chemical Co., St. Louis, Mo., and Tris (absolute grade) was obtained from Research Plus Laboratories, Denville, N.J. The reactions were incubated at 40°C for 90 min, after which 25- μ l portions were removed and precipitated by adding 200 μ l of 5% trichloroacetic acid. Precipitates were collected by filtration through Whatman GF/C filters, and the radioactivity was measured by counting in Scintaverse (Fisher Chemical Co.) with a model LS-233 scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

RNA purification. RNA transcripts were purified by first centrifuging the reaction mixture for 1 h at $85,000 \times g$ to remove the cores; then 1% sodium dodecyl sulfate (SDS) was added to the supernatant, and the preparation was incubated for 5 min at 37°C. The mixture was then phenol extracted three times, ether extracted twice, ethanol precipitated three times, lyophilized, suspended in water, and stored frozen at -70°C.

Electrophoresis. The products of the transcriptase reaction were separated by electrophoresis in slab gels

(13 by 16 cm) containing 1.5% agarose, 6 M urea, 0.04 M Tris (pH 7.4), 0.036 M NaH_2PO_4 , and 0.001 M EDTA. Electrophoresis was performed at a constant current of 30.0 mA for 24 h, after which the gel was rinsed to remove the urea, dried, and exposed to Kodak XR5 film for autoradiography. Electrophoresis of proteins was carried out in 10% polyacrylamide gels, as previously described (7).

RESULTS

Activation of the transcriptase. (i) Proteolytic activation of the three reovirus serotypes. Chymotrypsin treatment of purified reovirions cleaves the viral outer capsid polypeptides and activates the transcriptase (6). The conditions required for transcriptase activation are different for the three serotypes (13; see below). We first examined the behavior of the three serotypes under conditions previously shown to activate type 3 transcriptase reproducibly. When viruses were incubated in $1 \times$ SSC containing 100 μ g of chymotrypsin per ml for 30 min at 37°C, type 3 transcriptase was activated consistently (seven of seven attempts), type 2 transcriptase was activated sometimes (three of four attempts), and type 1 transcriptase was never activated (zero of seven attempts). A variety of treatments were studied in an effort to find reliable conditions which would activate the transcriptase of each serotype. The treatment conditions tested included heat (55 and 60°C), detergents (5% deoxycholate and 1% SDS), chemical denaturants (5 M urea), and other proteolytic enzymes (trypsin and proteinase K). The most reliable conditions utilized chymotrypsin plus heat; Fig.

FIG. 1. Optimization of conditions for transcriptase activation. The digestion conditions tested are listed for each reovirus serotype. The amount of incorporation was normalized within each serotype. The absolute levels of maximal incorporation were similar for all three serotypes.

TABLE 1. Genotypes of type 1 $\times$ type 3 recombinants tested for transcriptase activation

Clone	Genotype										Activation behavior
	L1	L2	L3	M1	M2	M3	S1	S2	S3	S4	
140	1 ^a	1	1	1	3	3	1	1	1	1	Type 3
F18	1	3	3	1	3	1	1	1	3	3	Type 3
H15	1	3	3	1	3	3	3	3	3	1	Type 3
H17	3	3	3	1	3	3	1	3	3	1	Type 3
H27	1	3	3	1	3	1	1		1	1	Type 3
H60	3	3	1	1	3	3	3	3	3	1	Type 3
F17	1	3	3	1	1	1	1	3	1	1	Type 1
G19	1	3	3	1	1	1	3	3	1	1	Type 1
H5	3	3	1	1	1	3	1	3	1	3	Type 1
H41	3	3	3	1	1	3	3	3	3	1	Type 1
G2	1	3	1	1	1	1	3	1	1	1	Variable
H9	3	3	1	3	1	1	3	3	3	3	Variable
H24	1	1	1	1	1	1	1	1	1	3	Variable

^a Numbers in the table indicate from which type of reovirus the genome segment of the recombinant clone originated.

1 shows the behaviors of the three serotypes with four different combinations of chymotrypsin and heat. Of all the conditions tried, type 3 transcriptase was activated optimally by chymotrypsin at 37°C; a higher temperature (i.e., 60°C) resulted in less activity. Although 37°C plus chymotrypsin produced significant activation of type 2 transcriptase in this experiment, the best activation was obtained with 1 min of treatment at 60°C. Type 1 transcriptase was not activated significantly by chymotrypsin at 37°C. Although significant activation was achieved by treatment for 1 min at 60°C, the best activation appeared after 10 min of treatment at 60°C; 30 min at 60°C resulted in reduced activity.

(ii) Identification of the double-stranded RNA

FIG. 2. Transcriptase activation behaviors of the type 1 (T-1) $\times$ type 3 (T-3) intertypic recombinants. The genotypes of the clones tested are listed in Table 1.

gene segment required for transcriptase activation. Since the transcriptases from types 1 and 3 displayed the greatest differences in the conditions needed for activation, we used intertypic recombinants (reassortants) derived from crosses between type 1 and type 3 (10, 11) to map the gene(s) responsible for activating the viral transcriptase. Table 1 shows the genotypes of the recombinants tested. Each recombinant was incubated under the conditions for optimal type 3 transcriptase activation (1 $\times$ SSC, 100 μ g of chymotrypsin per ml, 37°C, 30 min) and then examined for transcriptase activity. Under these conditions, the transcriptase of type 1 was not activated. Clones 140, H15, H17, F18, H27, and H60 behaved like type 3, whereas clones F17, G19, H41, and H5 behaved like type 1 (Fig. 2). These results clearly demonstrated that recombinants containing the M2 gene of type 3 always produced the pattern of transcriptase activation characteristic of type 3. This agreed with previous biochemical results of other workers (6), which showed that activation of the type 3 transcriptase was correlated with cleavage of the M2 gene product, as measured by visual inspection of stained gels. However, the presence of a type 1 M2 gene did not necessarily always produce the pattern of activation characteristic of type 1. For example, clones G2, H9, and H24 varied in their patterns of activation under these conditions (data not shown). Since the reason for this variability was not clear, additional biochemical experiments were performed.

(iii) Biochemical basis of resistance of type 1 transcriptase to activation by chymotrypsin. To gain a better understanding of the biochemical basis by which type 1 transcriptase resisted activation by chymotrypsin, we performed a time course study of digestion of ³⁵S-labeled type 1 at 37°C and analyzed the polypeptide products by SDS gel electrophoresis (Fig. 3).

FIG. 3. Effect of chymotrypsin digestion at 37°C on type 1. (A) Transcriptase activity present in type 1 virions after increasing digestion. (B) Infectivity of reovirus type 1 after increasing digestion. (C) SDS gel electrophoresis of the products of digestion of ³⁵S-labeled type 1. The arrow at the right indicates the cleavage product derived from polypeptide μ 1C.

The digestion of type 1 proceeded in a manner analogous to the digestion described previously for type 3 (6); i.e., σ 3 was digested first, followed by cleavage of μ 1C to an intermediate size. In contrast to type 3, the intermediate cleavage product of μ 1C from type 1 was not cleaved further. Under these conditions the transcriptase remained inactive, and the particles retained full infectivity. Control type 3 virions digested under these conditions showed the previously described cleavage of σ 3, followed by cleavage of μ 1C. μ 1C cleavage was accompanied by transcriptase activation and loss of infectivity (Fig. 4). Figure 5 shows the results of a time course study of proteolytic digestion of type 1 virions at 60°C. μ 1C was cleaved completely under these conditions within 1 min, the transcriptase was fully activated, and the infectivity of the particles decreased by more than 3 logs. Thus, type 1 was resistant to

proteolytic transcriptase activation at 37°C because the cleavage of the μ 1C polypeptide was blocked at an intermediate stage.

Characterization of the transcriptase activity.

(i) **Products of the type 1, 2, and 3 transcriptase reactions.** Reovirions contain a number of enzymatic activities which are able to convert ribonucleotides to acid-precipitable forms (5, 16). To confirm that the activity measured in our experiments was in fact the transcriptase, we labeled the products of the reactions with [α -³²P]UTP and analyzed them by gel electrophoresis. The RNA products of each reaction were purified and separated by electrophoresis on an agarose urea gel system, as described above (Fig. 6). Each serotype produced 8 to 10 species of the appropriate molecular weights in three size classes. In this gel system, the three serotypes displayed striking differences in the mobilities of the RNAs in all three size classes.

(ii) **Comparative properties of the transcriptases from reovirus types 1, 2, and 3.** Using

FIG. 4. Effect of chymotrypsin digestion at 60°C on type 1. (A) Symbols: ○, transcriptase activity; ●, infectivity. (B) SDS gel electrophoresis of the products of digestion.

FIG. 5. Effect of chymotrypsin digestion at 37°C on type 3. (A) Symbols: ○, transcriptase activity; ●, infectivity. (B) SDS gel electrophoresis of the products of digestion.

conditions that reproducibly activated the transcriptase of each of the three serotypes, we examined the properties of the transcriptases from the three serotypes in detail.

(a) **Divalent cations.** In agreement with the results of previous studies (6), we found that the type 3 transcriptase preferred Mg^{2+} to Mn^{2+} and that it displayed a sharp Mg^{2+} optimum at a concentration of 10 mM. As Fig. 7 shows, type 1 and 2 transcriptases behaved like type 3 transcriptase.

(b) **Monovalent cations.** The transcriptases from reovirus types 1, 2, and 3 all exhibited a slight preference for Na^+ over K^+ when they were assayed at the standard concentration of 70

mM. The ratios of activity in K^+ to activity in Na^+ were as follows: type 1 transcriptase, 0.79; type 2 transcriptase, 0.91; type 3 transcriptase, 0.87.

(c) **Temperature.** In agreement with the results of previous studies (6), type 3 transcriptase had a high temperature optimum. Although the three transcriptases showed some slight variability, their temperature activity curves were not distinguishably different; all displayed optimum activity at 45°C (Fig. 8).

(d) **pH.** The pH activity curve of the type 3 transcriptase was broad. As previously described, there was significant activity across the range from pH 8.0 to pH 9.0 (13, 15). Although the pH activity curves of type 1 and 2 transcriptases were similar to that of type 3 transcriptase, the three serotypes displayed a small but highly reproducible difference in this parameter; in every experiment the pH optimum of type 2 transcriptase was lower than that of type 3 transcriptase. This result was independent of the original stock of each serotype used, the batch of purified virus used, the source of the reagents used for the reaction, and the conditions of activation used. Figure 9 shows the results of a typical series of four experiments. In these ex-

FIG. 6. Agarose-urea gel electrophoresis of the products of the transcriptase reactions. Migration was from top to bottom. O, Origin; XC, location of xylene cyanol marker dye, which comigrated with single-stranded RNA molecules ~1,000 nucleotides long in this system; T1, type 1.

periments, type 2 transcriptase displayed an optimum at pH 8.5, whereas the optimum pH of type 3 transcriptase was 9.0. The activity of type 1 transcriptase (data not shown) was maximal and indistinguishable at both pH 8.5 and pH 9.0. In a series of reactions in which many stocks of reagents were used, the results were not always identical to those shown in Fig. 9. For example, the pH optima of type 2 and 3 transcriptases were sometimes 8.0 and 8.5, respectively, or occasionally they were 8.0 and 9.0, respectively, in an experiment. Thus, although the pH activity curves of type 2 and 3 transcriptases displayed some variability in their exact optimum values, the difference between them was highly reproducible. Figure 10 shows composite pH activity curves of type 2 and type 3 transcriptases and represents the pooled results of 14 experiments. These composite curves reflect the fact that both type 2 transcriptase and type 3 transcriptase could (in different experiments) display a pH optimum at pH 8.5. However, the pH optimum of type 2 transcriptase was never pH 9.0; likewise, the pH optimum of type 3 transcriptase was never pH 8.0. When paired T tests were used, the pH optimum of type 2 transcriptase was significantly different from that of type 3 transcriptase at a confidence level of 97%.

(iii) **Identification of the gene responsible for the transcriptase pH activity curve.** To determine which viral gene product(s) specified the pH activity curve, we used a number of type 2 $\times$ type 3 intertypic recombinants which carried various combinations of genes from reovirus types 2 and 3 (A. H. Sharpe, Ph.D. thesis, Harvard University, Cambridge, Mass., 1981).

FIG. 7. Divalent cation preference and optimization of the three reovirus transcriptases. The amount of incorporation was normalized within each serotype. Mg^{2+} concentrations below 5 mM or above 50 mM resulted in no activity, as did Mn^{2+} concentrations below 10 mM or above 50 mM. Symbols: ●, type 1, Mg^{2+} ; ▲, type 2, Mg^{2+} ; ■, type 3, Mg^{2+} ; ○, type 1, Mn^{2+} ; △, type 2, Mn^{2+} ; □, type 3, Mn^{2+} . T-1, Type 1.

FIG. 8. Temperature activity profiles of the three transcriptases. The amount of incorporation was normalized within each serotype. Symbols: ●, type 1; ■, type 2; △, type 3. T-1, Type 1.

The genotypes of the recombinants used are listed in Table 2.

The pH activity curve of each of the recombinants mimicked the curve of either type 2 or type 3; typical results are shown in Fig. 11. Certain recombinants, including clones 37, 169, 171, 225, and 106 behaved like the type 3 parental control in each experiment, with a pH optimum at either 8.5 or 9.0. Other recombinants (clones 204 and 269) behaved like the type 2 parental control in each experiment, with a pH optimum at either 8.0 or 8.5.

Clones 204 and 269 were the only recombinants which had the L1 gene of type 2 and also were the only recombinants which produced a transcriptase pH activity curve like that of type 2. All of the recombinants with the L1 gene of reovirus type 3 displayed a pH activity curve like type 3. No other genes were consistently associated with either pattern (type 2 or type 3); thus, the L1 gene specified the pH activity curve of the reovirus transcriptase.

DISCUSSION

We used a genetic method to study the activation and characteristics of the transcriptases of the three reovirus serotypes.

The three serotypes differ in the conditions under which their transcriptases are activated. Experiments with intertypic recombinants showed that this difference is specified by the M2 gene. Our assignment of the M2 gene product as the mediator of transcriptase activation agrees with and extends previous biochemical studies on type 3. Previous studies showed that transcriptase activation correlated with cleavage of the M2 gene product, as measured by visual inspection of stained gels. Our studies confirmed this and ruled out the involvement of other

FIG. 9. pH activity profiles of type 2 and 3 transcriptases. The amount of incorporation was normalized within each serotype. Symbols: ■, type 2; △, type 3. T-2, Type 2.

polypeptides, some of which are not resolvable due to comigration on gels or are not easily visible due to the small amounts present in virions. In addition, since the ratio of particles to PFU in purified reovirus stocks is typically about 100, these studies confirmed the functional importance of the M2 gene product within the viable population of transcriptionally active particles.

The behavior of reovirus type 1 in the presence of chymotrypsin also provides insight into the mechanism of reovirus uncoating. Studies with reovirus type 3 have shown that uncoating seems to be a two-step process in vivo and in vitro. The first step is dependent on an exoge-

nous protease and generates intermediate subviral particles, which are transcriptionally inactive. The second step takes place in the absence of proteolytic enzymes and results in transcriptionally active particles. Conversion of intermediate subviral particles from reovirus type 3 to transcriptionally active particles can be brought about easily by altering the salt conditions or by other mild treatments (2, 14).

The in vitro uncoating of reovirus type 1 seems to be analogous to the uncoating of reovirus type 3. The $\sigma 3$ protein of type 1 is digested with approximately the same kinetics as the $\sigma 3$ protein of type 3 (6; unpublished data). However, the second step of uncoating takes place much less readily. Transcriptionally inactive intermediate subviral particles from reovirus type 1 seemed to accumulate in vitro in all digestion conditions tested unless heat was also added. This pronounced block in the second step of uncoating makes reovirus type 1 an attractive candidate for the study of reovirus uncoating in vitro.

Reproducible conditions for activating the transcriptase of each serotype allowed us to make detailed comparisons of the three transcriptase reactions and their products. The single-stranded RNA products of all three reactions were synthesized in roughly inverse proportions to their lengths, and they displayed significant electrophoretic polymorphism in all size classes. This polymorphism implies that there are RNA nucleotide sequence differences among the serotypes and therefore suggested that the three transcriptases might differ in some measurable parameter.

The monovalent cation preferences, divalent cation preferences and optima, and temperature optima of type 1, 2, and 3 transcriptases were indistinguishable. These transcriptase properties, which are manifest in virion cores, provide a striking contrast to a number of virion properties which are known to be mediated by outer capsid components. Such outer capsid components mediate interactions between a virus and its environment both inside and outside hosts.

TABLE 2. Genotypes of type 2 $\times$ type 3 recombinants used to examine the basis of the reovirus pH activity curve

Clone	Genotype									
	L1	L2	L3	M1	M2	M3	S1	S2	S3	S4
37	3 ^a	3	3	2	2	2	3	2	2	3
106	3	2	3	2	2	2	3	3	3	3
169	3	3	2	2	3	2	2	3	3	2
171	3	3	2	3	3	3	3	3	3	3
204	2	2	2	2	2	2	2	2	2	3
225	3	3	3	2	2	3	3	3	3	3
269	2	3	2	2	2	2	3	3	2	3

^a See Table 1, footnote a.

For example, they specify the cell type to which the virus attaches. Accordingly, the outer capsid displays a high degree of structural and functional polymorphism in the three serotypes, which is reflected in their biological properties. In contrast, the transcriptionally active cores seem to be adapted to a single much less diverse environment, namely, the cytoplasm of mammalian cells.

However, the three transcriptases differ in their pH activity curves. Using intertypic recombinants, we determined that gene L1 specifies the pH activity curve. These data do not prove that the L1 gene product is the transcriptase itself, but only that this gene product determines the pH activity curve. However, assignment of the transcriptase to the L1 gene product would be consistent with other results. The L1 gene product is $\lambda 3$, a 140,000-dalton protein that is present in only a few (perhaps 10) copies per virion (8). All other RNA virus transcriptases are large (>100,000-dalton) proteins that are present in relatively few copies per virion (1).

Studies of reovirus type 3 with the transcriptase inhibitor pyridoxal phosphate have demonstrated that this inhibitor binds to polypeptides $\lambda 1$ and $\lambda 2$ within intact cores and that there are only about 10 high-affinity binding sites in each of the λ species. The binding of pyridoxal phosphate to these high-affinity sites was pH dependent, and the pH dependence of binding to $\lambda 2$ showed a striking similarity to the pH activity curve which we obtained for the type 3 transcriptase. However, this analysis of pyridoxal phosphate binding was based on electrophoresis of the virion polypeptides in a phosphate-urea gel system in which the minor component $\lambda 3$

FIG. 10. Composite pH activity profiles of type 2 and 3 transcriptases. The bars indicate the ranges of values obtained. Symbols: ■, type 2; △, type 3. T-2, Type 2.

comigrated with $\lambda 2$ (18). Therefore, we propose that the observed binding of pyridoxal phosphate to 10 high-affinity sites in $\lambda 2$ at a pH optimum of 9 in fact represents binding to each of the 10 copies of polypeptide $\lambda 3$, which is the transcriptase. Since we found no evidence that the L3 gene product $\lambda 1$ affects the transcriptase,

FIG. 11. pH activity profiles of transcriptases from reovirus types 2 and 3 and type 2 $\times$ type 3 intertypic recombinants. The genotypes of the recombinants tested are listed in Table 2. (A) Symbols: △, type 3; ●, clone 37; ○, clone 106. (B) Symbols: △, type 3; ■, clone 169; ●, clone 171; ○, clone 225. (C) Symbols: ■, type 2; ●, clone 204; ○, clone 269. T-3, Type 3.

the binding of pyridoxal phosphate to polypeptide $\lambda 1$ could implicate this protein in one of several other RNA synthetic activities present in reovirus cores.

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