Two defective forms of reverse transcriptase can complement to restore retroviral infectivity

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Retroviral DNA synthesis requires both the DNA polymerase and the RNaseH activities of reverse transcriptase (RT). To test whether two defective RTs—one carrying a mutation in the RNaseH domain and the other with a mutation in DNA polymerase—could work together to complete viral DNA synthesis, we generated phenotypically mixed virions of Moloney murine leukemia virus (M-MuLV) that contained two kinds of mutant RTs. One RNaseH catalytic site mutant complemented both tested DNA polymerase mutants and small amounts of intact viral DNA were generated. This demonstrates that retroviral DNA synthesis can be completed—albeit inefficiently—when DNA polymerase and RNaseH activities are provided by separate RT molecules. Other RNaseH mutants failed to complement, suggesting that some aspects of the RNaseH domain are essential to RT's DNA polymerase function. Phenotypically mixed virions were also used to demonstrate that RT and integrase (IN) can be provided by separate polyprotein precursors and complete the early stages of retroviral replication.

Key words: complementation/retrovirus/reverse transcription/RNaseH

Introduction

Reverse transcriptase follows an intricate series of steps to synthesize a double-stranded DNA copy of retroviral genomic RNA (Gilboa *et al.*, 1979). This critical process in the retroviral replication cycle requires the interplay of reverse transcriptase's DNA polymerase activity, which can use either RNA or DNA as a template, and its RNaseH activity, which degrades the RNA strand of the RNA–DNA hybrids that are formed as replication intermediates (Skalka and Goff, 1993).

For all retroviral RTs that have been characterized, the DNA polymerase and RNaseH active sites are physically linked within a single polypeptide (Goff, 1990). The catalytic sites for these two activities lie in physically separable domains of RT and mutations can be made in one or the other domain that disrupt the resident activity but do not impair basic catalytic function of the other activity (Tanese and Goff, 1988). Mutations that destroy either the DNA polymerase or the RNaseH activity of RT render retroviruses non-infectious. Virions that harbor such defective forms of RT generate characteristic abortive reverse transcription products that reflect the failure of the mutant enzymes to

carry out all the steps of the reverse transcription process. For example, the generation of retroviral DNA by many RNaseH mutants is aborted after synthesis of the initial minus strand DNA intermediate, since the absence of RNaseH activity prevents the nucleolytic removal of the template strand that is required before further DNA synthesis can proceed (Tanese *et al.*, 1991).

Examining RNA cleavage products generated by RNaseH action during reverse transcription *in vitro* suggests that RT's polymerase and RNaseH activities can function coordinately, with the two catalytic sites of RT separated by a distance equal to ~15–19 bp of RNA–DNA hybrid (Oyama *et al.*, 1989; Luo and Taylor, 1990; Kati *et al.*, 1992). Some researchers have suggested that DNA synthesis and template degradation may occur contemporaneously and be performed by a single RT molecule during retroviral DNA synthesis (Schatz *et al.*, 1990; Peliska and Benkovic, 1992). However, in reactions *in vitro*, RT's two activities can function separately (Champoux, 1993); that is, RNaseH cleavages can occur in the absence of DNA polymerase activity and DNA polymerization can take place independently of RNaseH degradation. Kinetic measurements of the relative rates of RT's DNA polymerase and RNaseH activities *in vitro* suggest that DNA synthesis can proceed largely independently of RNaseH action, suggesting that the two activities might function sequentially and involve separate RT molecules (Kati *et al.*, 1992). It has remained unclear whether the two activities must be coupled during retroviral replication within infected cells (DeStefano *et al.*, 1991; Hostomsky *et al.*, 1991; Champoux, 1993).

To address the issue of DNA polymerase and RNaseH coupling during retroviral replication, we generated phenotypically mixed virions of Moloney murine leukemia virus (M-MuLV) that contained a mixture of DNA polymerase and RNaseH-mutant forms of RT. These mixed virions were shed by producer cells that expressed two genetically distinct integrated proviruses. By providing a mixture of defective forms of RT within individual viral particles, we tested whether DNA synthesis could be completed when RNaseH mutant RTs with functional DNA polymerase activity synthesized DNA, and DNA polymerase mutants with functional RNaseH provided the RNA-degrading functions necessary in replication.

Results

Generating phenotypically mixed virions

The experiments presented here used phenotypically mixed M-MuLV virions engineered so that each virion contained a mixture of defective pol gene products. The phenotypically mixed virion producer cell lines were constructed by serially transfecting NIH3T3 cells with two different mutant proviruses (see Materials and methods). Introducing the proviral clones separately was necessary to ensure that protein complementation was assayed in the absence of any

Fig. 1. Positions of pol gene mutations used in this study. M-MuLV proviral DNA is shown at the bottom; mutation sites are indicated on an enlarged portion of the pol gene showing the coding regions for RT and part of IN. A4, M4 and 5247 are linker insertion mutations in RT DNA polymerase, RT RNaseH and IN coding regions, respectively (Tanese and Goff, 1988; Roth *et al.*, 1990). DD224NN is a substitution of two asparagine residues for the aspartic acid residues at positions 224 and 225. This mutation destroys a motif, YxDD, which is conserved in many RNA-dependent polymerases and abolishes DNA polymerase in this and other RTs (Doolittle *et al.*, 1989; Lowe *et al.*, 1991). D524N introduces an Asn in place of Asp in an RNaseH domain residue postulated to function in catalysis (Repaske *et al.*, 1989; Yang *et al.*, 1990; Blain and Goff, 1993; Telesnitsky and Goff, 1993a); ΔC and Δ5401 are deletions in the RT RNaseH domain and IN, respectively (Schwartzberg *et al.*, 1984; Telesnitsky *et al.*, 1992); H7 is a frame-shift mutation which results in the expression of a single DNA polymerase domain form of RT, analogous to ΔRH RT (Tanese and Goff, 1988; Telesnitsky *et al.*, 1992; Telesnitsky and Goff, 1993a).

illegitimate recombination between viral genomes that might have occurred if the different proviruses were introduced simultaneously. These successive transfections resulted in the stable integration of two different mutant proviruses into the producer cells' chromosomes, and the expression and co-assembly of two genetically distinct gag-pol polypeptide precursors. Since ~100 gag-pol precursors contribute to the formation of every retroviral particle (Varmus and Brown, 1989), the co-expression within single cells of two proviruses with different pol gene mutations yielded phenotypically mixed virions, each of which contained a mixture of the two different defective pol proteins. Each of the pol mutations used in this study renders the virus non-infectious; these mutations are summarized in Figure 1. At least three independently established virion producer lines were used to test complementation for each pair of defective proviruses, and all yielded the same results.

Complementation by reverse transcriptase mutants

Complementation between mutant RTs was defined to mean that together two defective enzymes would complete the synthesis of integration-competent DNA, even though individually, none of the mutant RTs used in this study was able to synthesize viral DNA. The basis of our complementation assay is presented in Figure 2 and is summarized as follows. Alone, a DNA polymerase-defective form of RT synthesizes no viral DNA. Alone, RNaseH mutants synthesize the first replication intermediate, known as minus strand strong stop DNA, but are unable to complete minus strand DNA synthesis or initiate plus strand DNA synthesis. However, if a DNA polymerase mutant with functional RNaseH could act after the RNaseH mutant and degrade the nascent RNA–DNA hybrid, then the process of reverse transcription might proceed. Complementation would occur if the co-packaged DNA polymerase and RNaseH mutant RTs could act together to carry out all the steps required in the generation of retroviral DNA.

Fig. 2. Schematic representation of the complementation assay. The steps involved in the synthesis of the first minus strand of DNA are depicted as they would occur within a phenotypically mixed virion that has encapsidated two genetically distinct genomic RNAs. RNA is represented with light lines; DNA with bold lines. Molecules of RT are represented by peanut-shaped objects. Both mutant RTs are present in the virion at all stages but for simplicity only the form of RT that is active in a given step is depicted. (A) First, RNaseH mutant RT with DNA polymerase activity synthesizes minus strand strong stop DNA (indicated by bold arrow), the first intermediate in retroviral DNA synthesis, which is initiated from the 3' end of a primer tRNA partially annealed to the genomic RNA at the PBS (primer binding site), and proceeds until the 5' end of the RNA is reached. (B) DNA polymerase mutant RT with functional RNaseH then degrades the RNA strand of the RNA–DNA hybrid formed in panel A. This exposes minus strand R (for 'repeated at both ends of genomic RNA') region DNA, which can then base-pair with complementary sequences at the 3' end of the genomic RNA, a step known as the first strand transfer, or 'jump', in reverse transcription (Gilboa *et al.*, 1979; Telesnitsky and Goff, 1993b). (C) After the first strand transfer, minus strand DNA synthesis proceeds. If template switching during reverse transcription occurs between the sites of the two RT mutations, a wild-type DNA will be synthesized.

Recombination during reverse transcription formed the basis of our assay for the generation of a complete retroviral DNA. Each provirus co-expressed in our producer cells encoded packageable genomic RNA. Thus, since retroviruses co-package two genomic RNAs per viral particle, a fraction of the phenotypically mixed virions shed

Fig. 3. (a) Summary of infection results. NIH3T3 cells were infected and passaged as described in Materials and methods. The mutants are as described in Figure 1. Except in the case of RT DNA polymerase mutants and the titration of wild-type virus, cells were infected with equivalent amounts (as standardized by RT DNA polymerase assays) of each virus stock. The gray tones represent relative levels of RT DNA polymerase activity observed in assays such as that presented in panel b. The time scale at the top of the figure represents days after infection. The sections marked 'RT + IN mutant pairs' and 'DNAP + RH mutant pairs' present data for phenotypically mixed virions containing RT and IN or DNA polymerase and RNaseH mutants, respectively. Throughout 6 weeks of passage, no RT DNA polymerase activity was detectable in the culture media of cells infected with those mixed virions that are indicated as displaying no complementation. (b) Representative data used to measure viral spread by RT DNA polymerase assay. Culture media from NIH3T3 cells infected with the indicated virus stocks were harvested and assayed for RT DNA polymerase activity. Culture supernatants with high levels of virus generate dark spots in this assay. Those with lower levels yield lighter spots and assays of media without wild-type virus result in blank regions. Virus designations are as in panel a.

by these cells not only contained a mixture of mutant RT proteins, but were genetically heterozygous as well. Genetic recombination that results from template switching during reverse transcription can be detected in the DNA of virions that have co-packaged two genetically distinguishable RNAs; when markers are 1 kb apart, it has been calculated that ~2% of progeny DNAs can be recombinant (Hu and Temin, 1990a,b). Thus, in our experiments, if template switching during reverse transcription occurred between the sites of the two mutations, then wild-type viral DNA would be produced. The resulting fully infectious virus would rapidly spread through the culture and would be detectable by assaying the culture media for RT DNA polymerase activity. In contrast, if DNA polymerase- and RNaseH-defective forms of RT failed to complement, then DNA synthesis would be aborted prior to the completion of integration-competent DNA, since the series of steps involved in retroviral DNA synthesis requires both activities. No complete DNA would be formed, no recombination could occur and no viral spread would result. Thus, the culture media of cells infected with mixed virions containing non-

complementing RT mutants would remain virus-free and would assay negative for RT DNA polymerase activity.

Protein complementation between RNaseH- and DNA polymerase mutant forms of RT was tested using several types of phenotypically mixed virions, each of which contained a DNA polymerase mutant and also an RNaseH mutant. In such mixed virions, one RNaseH mutant, D524N, which contained a single amino acid substitution in a putative RNaseH catalytic site residue (Repaske *et al.*, 1989; Kanaya *et al.*, 1990; Blain and Goff, 1993; Telesnitsky and Goff, 1993a), complemented two different DNA polymerase mutants, as assayed by the generation of wild-type virus in the culture media of cells infected with phenotypically mixed virions containing both D524N and a DNA polymerase mutant form of RT. Other RNaseH mutants failed to complement the DNA polymerase mutants. For all of the RT DNA polymerase and RNaseH mutants used in this study, virions containing any one of the RT mutants individually were completely defective and did not generate any full-length viral DNA or induce viral spread in infected cells, even after 6 weeks of passage (Figure 3a and data not

shown). The complementation results for all pol mutant pairs tested in this study are summarized in Figure 3a.

Complementation could also be tested by direct examination of the viral DNA synthesized in infected cells. Phenotypically mixed virions containing both D524N and DNA polymerase mutant forms of RT produced full-length viral DNA, whereas virions containing only one of the two mutant RTs did not. However, the amount of full-length preintegrative DNA synthesized during the first round of infection by the D524N RNaseH mutant/DNA polymerase mutant mixed virions was drastically reduced relative to wild-type (Figure 4a). This suggests that the overall delay in the production of wild-type virus in cells infected with these phenotypically mixed virions (Figure 3) was caused both by the need for recombination to generate wild-type DNA and by the relative inefficiency of the complementation process, as indicated by the reduced frequency with which full-length DNA was generated. Most of the RNaseH mutants we tested failed to complement the DNA polymerase mutants and no preintegrative DNA was generated, as assayed by an absence of detectable viral spread during 6 weeks of passage of cells infected with DNA polymerase mutant/RNaseH mutant mixed virions.

Complementation of reverse transcriptase and integrase mutants

Phenotypically mixed virions were also used to test whether mutations in the RT and integrase (IN) coding regions of the retroviral pol gene could complement *in trans*. IN is an enzyme required for retroviral DNA integration and like RT, IN is processed from the pol portion of gag-pol precursors (Varmus and Brown, 1989). RT and IN appear to maintain a physical association after their proteolytic separation (Hu *et al.*, 1986) and it was unknown prior to these experiments whether IN and RT could be provided by separate gag-pol precursors. To test whether functional RT from one kind of gag-pol precursor could synthesize viral DNA and functional IN derived from the other co-packaged class of gag-pol precursors could then integrate this DNA, we produced phenotypically mixed virions assembled from an assortment of gag-pol precursors, some of which had functional RT and mutant IN and others of which had mutant RT and functional IN. We observed wild-type viral spread, diagnostic of protein complementation and the occasional generation of wild-type viral DNA via template switching during reverse transcription, in infections by phenotypically mixed virions containing all tested pairs of RT and IN mutants (Figure 3). Our results demonstrated that RT and IN could be provided by separate precursors and complement *in trans* to complete the early stages of retroviral replication.

Examining the restriction patterns of preintegrative DNA generated during the first round of infection by one such mixed virion demonstrated that most of the DNAs retained the genotype of one of the two parental proviruses (Figure 4b). This demonstrated that the integrated proviruses in the producer cell lines had not recombined since wild-type RNA was not detected. The time of appearance of detectable virus after infection with these mixed virions suggested that during the first round of viral replication, wild-type viral DNA was synthesized and integrated at ~1/50th the frequency at which it was generated by wild-type virus. This delay in virus appearance approximates the rate at which wild-type DNA would be predicted to result from template

Fig. 4. (a) Preintegrative DNA formed during infection with DNA polymerase/RNaseH mutant phenotypically mixed virions. Virus-free Rat2 cells were infected with equivalent amounts (as standardized by RT DNA polymerase assays) of wild-type virus, phenotypically mixed viruses containing DNA polymerase DD224NN mutant and the RNaseH D524N mutant, or phenotypically mixed viruses containing DNA polymerase mutant A4 and the RNaseH D524N mutant, and subjected to Southern blot analysis. Marker sizes are presented in kbp on the right. (b) *EcoRI* digestion patterns of preintegrative DNA formed during infection with RT/IN mutant phenotypically mixed virions. Wild-type M-MuLV contains no *EcoRI* sites and both RT mutant A4 and IN mutant IN5247 are *EcoRI* linker insertion mutants. Lane 1, wild-type DNA. Uncleaved preintegrative linear proviral DNA migrates at 8.8 kb; one- and two-LTR circular forms were also uncleaved, did not transfer efficiently to the hybridization membrane, and hence are not visible in this Southern blot. Lane 2, IN 5247 DNA. *EcoRI* cleavage of the linear proviral DNA yielded bands of 5.2 and 3.6 kb. Linearization of one- and two-LTR circular forms generated 8.2 and 8.8 kb bands, respectively. Lane 3, DNA from infection with A4/IN 5247 phenotypically mixed virions. In addition to the bands produced from digestion of DNA synthesized using IN 5247 RNA as a template, A4 RNA-templated products are observed: linear A4 proviral DNA yielded bands of 3.2 and 6.6 kb. In this and other restriction analyses not shown, no recombinant DNAs that contained either both or neither of the mutations were detectable. Marker sizes are presented in kbp on the right.

switching during reverse transcription between the sites of the RT and IN mutations, and suggests that the delay in wild-type virus appearance was largely dictated by normal retroviral recombination frequencies. Apparently, the presence of inactive RT and IN in these phenotypically mixed virions did not drastically impair the function of the active RT and IN proteins.

Discussion

These results show that retroviral DNA synthesis can be completed when the DNA polymerase and RNaseH activities of reverse transcriptase are provided by separate RT molecules. This resolves the long-standing question of whether RT's DNA polymerase and RNaseH activities can complete DNA synthesis if they are physically uncoupled by demonstrating that covalent linkage of these two activities is not absolutely necessary. However, at least with the pairs of mutants used in this study, retroviral DNA synthesis is much less efficient when DNA polymerase and RNaseH

activities are provided by separate molecules than when the two activities reside within a single polypeptide, as in the wild-type enzyme.

The RNaseH activity of reverse transcriptase performs several sequence-specific functions in retroviral replication, such as the generation of the correct primer for plus-strand DNA synthesis and the accurate removal of the primer tRNA (Champoux, 1993). The fact that two different DNA polymerase null mutants—one with a missense mutation and the other a four amino acid insertion—were both able to complement the D524N RNaseH mutant demonstrates that the complementation we observed is not allele specific. Although these assays do not allow us to determine the relative contributions of lesions in the DNA polymerase versus RNaseH to the overall inefficiency of complementation, our results indicate that both the DNA polymerase mutants used in this study are at least weakly capable of performing all the RNaseH functions required during retroviral replication. On the other hand, several RNaseH mutants failed to complement either DNA polymerase mutant. Thus, whereas all the RNaseH mutants used in this study retained wild-type levels of DNA polymerase activity in simple nucleotide incorporation assays (Tanese and Goff, 1988; Telesnitsky *et al.*, 1992; Telesnitsky and Goff, 1993a), most mutants apparently failed to provide the DNA polymerase functions in a form suitable for retroviral replication.

We have recently shown that RNaseH domain mutations can affect RT's DNA polymerase activity by altering the interaction between RT and its primer template (Telesnitsky and Goff, 1993a). Such effects may be responsible for the failure of some RNaseH mutants to complement in the assays presented here. Some RT RNaseH domain mutations affect the processivity of DNA polymerization. Also, some RNaseH domain mutations interfere with the ability of M-MuLV RT to form dimers. Although M-MuLV is monomeric in solution, it appears to dimerize when productively bound to primer template (Telesnitsky and Goff, 1993a). The Δ C RNaseH deletion mutant is unable to form dimers and is also less processive in DNA synthesis than wild-type RT. In the experiments presented here, Δ C RT failed to complement DNA polymerase mutant RTs. The RNaseH mutant form of RT that did complement, D524N, can dimerize. However, complementation requires more than the ability to dimerize, since the M4 RNaseH insertion mutant can form RT dimers but failed to complement DNA polymerase mutant RTs.

Our complementation results suggest that various pairs of mutant RTs differ in their ability to synthesize infectious DNA *in vivo* and demonstrate that retroviral DNA in some settings can be synthesized when DNA polymerase and RNaseH activities are provided by separate polypeptides. However, the mechanism by which DNA polymerase and RNaseH mutants complement remains unclear. If each subunit of the M-MuLV RT dimer contributes one of RT's two enzymatic activities during retroviral replication, then the DNA polymerase mutants and the D524N RNaseH mutant may complement as partners in a heterodimer. Genetic analyses of the HIV RT heterodimer have demonstrated that both DNA polymerase and RNaseH activities are provided by only the larger of its two subunits (Le Grice *et al.*, 1991; Hostomsky *et al.*, 1992) and the three-dimensional structure of HIV RT shows that the HIV

enzyme is an asymmetric dimer, with the conformation of the smaller HIV RT subunit different from the corresponding portion of the larger, catalytically active subunit (Kohlstaedt *et al.*, 1992). Thus, if both activities of the M-MuLV RT dimer are provided by only one subunit—as is true of HIV-1 RT—then it is likely that the complementation we observe occurs via sequential action of the DNA polymerase and RNaseH mutants. Analysis of the functional symmetry of the M-MuLV RT dimer will help resolve the mechanism of complementation in the experiments presented here.

Materials and methods

Cells and viruses

Rat2 cells, NIH3T3 cells and derivative producer cell lines were grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum (Gibco). Viral supernatants (5 ml) were harvested from 10 cm plates of 90% confluent producer cells at 12 h intervals, filtered using 0.45 μ M filters (Gelman), dispensed and stored at -80°C prior to use. Neomycin-resistant cells were selected by growth in media containing 400 $\mu\text{g}/\text{ml}$ G418 (Gibco) and histidinol-resistant cells were selected in 5 mM histidinol (Sigma).

Construction of producer cell lines

Phenotypically mixed virion producer cell lines were established as follows. NIH3T3 cells were co-transfected by the calcium phosphate method (Wigler *et al.*, 1979) with a mixture of pSV2-neo DNA (Southern and Berg, 1982) and a given mutant derivative of the M-MuLV provirus-containing plasmid, pNCA (Colicelli and Goff, 1988), harboring an RT DNA polymerase region mutation. G418-resistant colonies were single-cell cloned and screened for resistance to superinfection (Granowitz *et al.*, 1991) and culture supernatants were tested to confirm the lack of detectable RT DNA polymerase activity (Goff *et al.*, 1981). Superinfection-resistant clones were assayed for the production of RT-defective virions by immunoprecipitation of mature viral proteins from the culture supernatant (Roth *et al.*, 1990). Cell lines thus determined to express the first defective provirus were subjected to a second round of co-transfection with a mixture of pSV2-his DNA (Hartman and Mulligan, 1988) and the second defective provirus. Histidinol-resistant clones were screened for RT DNA polymerase activity in the culture media. At least three independently derived clones from each of the secondary co-transfections were used in tests for complementation.

Complementation assays

The ability of the proteins in phenotypically mixed virions to complement were scored as follows. In the initial screens, 10% confluent, virus-free NIH3T3 cells were infected with undiluted, filtered culture supernatants harvested from 90% confluent virion producer lines. When the infected cells became confluent, a sample of the culture supernatant was collected and the cells were passaged at a 1:10 dilution. The infected cells were passed in this way for 6 weeks. Those which generated RT DNA polymerase activity in the culture supernatant during this period were subjected to a secondary test. In the secondary test for complementation, filtered supernatants from producer lines were normalized such that the same amount of RT DNA polymerase activity was used in each infection and cells were passaged at 1:5 dilutions and culture supernatants were assayed for RT DNA polymerase activity.

RT DNA polymerase activity assay

The RT DNA polymerase activity of viral supernatants was measured using a standard assay (Goff *et al.*, 1981) that measures dTMP incorporation using an oligo(dT) poly(A) primer template.

Infection of cells and preparation and analysis of viral DNA

Rat2 cells were infected with viral supernatants (6 ml of virus per 15 cm plate) in the presence of 8 $\mu\text{g}/\text{ml}$ polybrene for 2 h at 37°C . Cells were lysed 12 h post-infection, and low molecular weight DNA was isolated (Hirt, 1967). This low molecular weight DNA, which is enriched for viral sequences, was either subjected to restriction analysis or applied directly to 0.7% agarose gels, blotted and probed (Southern, 1975) with a viral DNA probe.

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References

- Blain, S.W. and Goff, S.P. (1993) *J. Biol. Chem.*, in press.
- Champoux, J.J. (1993) In Skalka, A.M. and Goff, S.P. (eds), *Reverse Transcriptase*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 103–117.
- Colicelli, J. and Goff, S.P. (1988) *J. Mol. Biol.*, **199**, 47–59.
- DeStefano, J.J., Buiser, R.G., Mallaber, L.M., Myers, T.W., Bambara, R.A. and Fay, P.J. (1991) *J. Biol. Chem.*, **266**, 7423–7431.
- Doolittle, R.F., Feng, D.-F., Johnson, M.S. and McClure, M.A. (1989) *Q. Rev. Biol.*, **64**, 1–30.
- Gilboa, E., Mitra, S.W., Goff, S.P. and Baltimore, D. (1979) *Cell*, **18**, 93–100.
- Goff, S.P. (1990) *J. AIDS*, **817–831**.
- Goff, S.P., Traktman, P. and Baltimore, D. (1981) *J. Virol.*, **38**, 239–248.
- Granowitz, C., Colicelli, J. and Goff, S.P. (1991) *Virology*, **183**, 545–554.
- Hartman, S.C. and Mulligan, R.C. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 8047–8051.
- Hirt, B. (1967) *J. Mol. Biol.*, **26**, 365–371.
- Hostomsky, Z., Hostomska, Z., Hudson, G.O., Woomaw, E.W. and Nides, B.R. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 1148–1152.
- Hostomsky, Z., Hostomska, Z., Fu, T.-B. and Taylor, J. (1992) *J. Virol.*, **66**, 3179–3182.
- Hu, S.C., Court, D.L., Zweig, M. and Levin, J.G. (1986) *J. Virol.*, **60**, 267–274.
- Hu, W.-S. and Temin, H.M. (1990a) *Proc. Natl Acad. Sci. USA*, **87**, 1556–1560.
- Hu, W.-S. and Temin, H.M. (1990b) *Science*, **250**, 1227–1233.
- Kanaya, S., Kohara, A., Miura, Y., Sekiguchi, A., Iwai, S., Inoue, H., Ohtsuka, E. and Ikehara, M. (1990) *J. Biol. Chem.*, **265**, 4615–4621.
- Kati, W.M., Johnson, K.A., Jerva, L.F. and Anderson, K.S. (1992) *J. Biol. Chem.*, **267**, 25988–25997.
- Kohlstaedt, L.A., Wang, J., Friedman, J.M., Rice, P.A. and Steitz, T.A. (1992) *Science*, **256**, 1783–1790.
- Le Grice, S.F.J., Naas, T., Wohlgensinger, B. and Schatz, O. (1991) *EMBO J.*, **10**, 3905–3911.
- Lowe, D.M., Parmer, V., Kemp, S.D. and Larder, B.A. (1991) *FEBS Lett.*, **282**, 231–234.
- Luo, G.X. and Taylor, J. (1990) *J. Virol.*, **64**, 4321–4328.
- Oyama, F., Kikuchi, R., Crouch, R.J. and Uchida, T. (1989) *J. Biol. Chem.*, **264**, 18808–18817.
- Peliska, J.A. and Benkovic, S.J. (1992) *Science*, **258**, 1112–1118.
- Repaske, R., Hartley, J.W., Kavlick, M.F., O'Neill, R.R. and Austin, J.B. (1989) *J. Virol.*, **63**, 1460–1464.
- Roth, M.J., Schwartzberg, P., Tanese, N. and Goff, S.P. (1990) *J. Virol.*, **64**, 4709–4717.
- Schatz, O., Mous, J. and Le Grice, S.F.J. (1990) *EMBO J.*, **9**, 1171–1176.
- Schwartzberg, P., Colicelli, J. and Goff, S.P. (1984) *Cell*, **37**, 1043–1052.
- Skalka, A.M. and Goff, S.P. (eds) (1993) *Reverse Transcriptase*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Southern, E.M. (1975) *J. Mol. Biol.*, **98**, 503–517.
- Southern, P.J. and Berg, P. (1982) *J. Mol. Appl. Genet.*, **1**, 327–341.
- Tanese, N. and Goff, S.P. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 1777–1781.
- Tanese, N.T., Telesnitsky, A. and Goff, S.P. (1991) *J. Virol.*, **65**, 4387–4397.
- Telesnitsky, A. and Goff, S.P. (1993a) *Proc. Natl Acad. Sci. USA*, **90**, 1276–1280.
- Telesnitsky, A. and Goff, S.P. (1993b) In Skalka, A.M. and Goff, S.P. (eds), *Reverse Transcriptase*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 49–83.
- Telesnitsky, A., Blain, S.W. and Goff, S.P. (1992) *J. Virol.*, **66**, 615–622.
- Varmus, H. and Brown, P. (1989) In Berg, D.E. and Howe, M.M. (eds), *Mobile DNA*. American Society for Microbiology, Washington DC, pp. 53–108.
- Wigler, M., Sweet, R., Sim, G.K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S. and Axel, R. (1979) *Cell*, **16**, 777–785.
- Yang, W., Hendrickson, W.A., Crouch, R.J. and Satow, Y. (1990) *Science*, **249**, 1398–1405.

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