Review Letter

DNA LIGASES OF EUKARYOTES

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1. Introduction

DNA ligases [poly(deoxyribonucleotide): poly(deoxyribonucleotide) ligase EC 6.5.1] join single-strand breaks in double-stranded DNA by catalyzing the formation of phosphodiester bonds. These enzymes were first discovered in bacteria and phage-infected bacteria, and the properties of the well characterized microbial DNA ligases have been reviewed [1–5].

DNA ligases act in collaboration with DNA polymerases during DNA replication and DNA repair. It has been shown that the DNA polymerases of mammalian cells differ considerably from the DNA polymerases of micro-organisms, and several reviews on the properties of mammalian DNA polymerases have recently appeared [6–11]. The mammalian DNA ligases also differ in many respects from bacterial DNA ligases, but the properties of the DNA ligases of higher organisms have not been previously reviewed. The present paper provides a summary of the features of this group of enzymes.

2. General properties of eukaryote DNA ligases

DNA ligases require a divalent metal ion and either ATP or NAD^+ as a cofactor. The enzymes in bacteria (*Escherichia coli, Bacillus subtilis*) require NAD^+ [1–5,12] while ATP-dependent ligases are induced by several *E. coli* bacteriophages such as T4 and T7 [1–5,13]. The DNA ligases of mammalian cells and plant cells uniformly seem to employ ATP as a cofactor, and no NAD^+-dependent DNA ligase from a higher organism has so far been described.

Bacterial cells appear to contain only a single DNA ligase. In contrast, mammalian cells have two different DNA ligases [14–19], which are serologically unrelated and respond in a different fashion to cell proliferation. These two mammalian enzymes have been called either 'DNA ligase I' and 'DNA ligase II', or 'cytoplasmic DNA ligase' and 'nuclear DNA ligase'. The properties of DNA ligase I and cytoplasmic DNA ligase appear to be identical, and similarly the DNA ligase II and nuclear DNA ligase would seem to be the same enzyme. In addition, small amounts of a DNA ligase activity with properties similar or identical to those of DNA ligase II have been found in isolated mitochondria [20]. The subcellular distributions of these enzymes have so far only been investigated in aqueous sucrose solutions. This fractionation method has been shown to be unreliable from the point of view that artificial leakage of nuclear enzymes into the cytoplasm may occur during the isolation of nuclei [21,22]. Further, while most of the DNA ligase I activity is recovered in the cytoplasmic fraction, a significant amount of DNA ligase I is also found in the nuclei [15]. Consequently, both the mammalian DNA ligases may well be primarily localized to the nuclei in vivo, and here we employ the designations DNA ligase I and II.

3. Mammalian DNA ligase I

The levels of DNA ligase activity are much lower in extracts from mammalian cells than from bacteria, and DNA ligase is most easily detected in extracts from tissues containing a high proportion of dividing cells. The dominating DNA ligase activity in

such organs is DNA ligase I, and consequently most studies on a mammalian DNA ligase have dealt with the properties of this enzyme. It has been partly purified and characterized from rabbit [23], calf [14,15,24–26], human [27,28], mouse [29], rat [16,19] and hamster [17,30] cells and tissues, but a homogenous enzyme is not yet available. A rabbit antiserum against 1000-fold purified calf thymus DNA ligase I has been shown to equally effectively inhibit the enzyme from other calf tissues as well as the corresponding enzyme from human, mouse, rat and rabbit cells [15,19]. DNA ligase I from several species has a broad pH optimum at pH 7.8 with 50% of its maximal activity retained at about pH 6.3. The optimal Mg^{2+} concentration is 0.010–0.015 M, and Mg^{2+} can be replaced by Mn^{2+} but not by Ca^{2+}. The activity of DNA ligase I at optimal Mg^{2+} concentration is not further enhanced by the addition of NH_4^+ ions, in contrast to the *E. coli* DNA ligase. The enzyme is relatively sensitive to inhibition by neutral salts, and the addition of 0.1 M versus 0.2 M NaCl to standard reaction mixtures causes a 50–75% versus an 85–97% inhibition [14,16,17]. DNA ligase I from rodent tissues is somewhat more sensitive than the calf thymus enzyme in this respect [31]. Reducing agents such as β-mercaptoethanol or dithiothreitol are necessary for optimal activity, and treatment with parahydroxymercuribenzoate or *N*-ethylmaleimide in the absence of such agents causes essentially complete inhibition. The enzyme functions effectively at quite low ATP concentrations, and has an apparent K_M of 2×10^{-7}–1.5×10^{-6} [23,24,29,30]. Nevertheless, there is an absolute requirement for ATP, and NAD^+ or GTP can not serve as alternate cofactors. The purified human enzyme appears to be unspecifically stimulated by the addition of other proteins present in boiled crude extracts to reaction mixtures [27,28], but it seems unlikely at present that DNA ligase I has an additional cofactor besides ATP. The enzyme is not significantly inhibited by either 10^{-3} M hydroxyurea or 10^{-2} M caffeine [31]. On heating in a buffer containing 0.3 M KCl, 0.05 M Tris-HCl (pH 7.4 at 20°C), 10^{-3} M dithiothreitol, and 10^{-3} M EDTA, DNA ligase I is 50% inactivated after 5 min at 52°C and retains full activity after 5 min at 45°C [14,19].

DNA ligase I is a large enzyme. Mol. wt. estimates from sedimentation velocity and gel filtration data are 220 000 for the mouse enzyme [29], 175 000 for the calf enzyme [14], and 190 000 for the human enzyme [28]. Falaschi and co-workers [28] found that in freshly prepared human cell extract, the DNA ligase I activity is present in such a high mol. wt. form. On prolonged incubation of the crude extracts, or during enzyme purification, a new active form of the enzyme appeared of one-half the original mol. wt. Similar results have also been obtained with the calf thymus enzyme. These two forms of DNA ligase I are equally inhibited by antibodies against the enzyme and are heat-inactivated at the same rate [15]. It is not known if the smaller form of DNA ligase I is due to dissociation of a dimeric form of the enzyme into a monomeric form, or to dissociation of another strongly bound protein from the ligase. There are precedents for both models. Thus, the *E. coli* exonuclease I has usually been isolated as a monomeric species of a mol. wt. of 70 000, but when a more rapid and gentle preparative procedure is employed, the enzyme is instead recovered as a dimer of mol. wt. 140 000. The monomer and dimer forms are equally active, are inhibited in an identical fashion by antiserum against the enzyme, and have the same catalytic properties [32]. On the other hand, several proteins involved in DNA replication may interact specifically with each other, and the products of phage T4 genes 44 and 62 have been co-purified as a tightly bound complex of the two proteins [33]. It seems less likely that the small form of mammalian DNA ligase I is due to limited proteolytic digestion, because the conversion of the highly purified calf thymus enzyme to a smaller form occurs more rapidly in a buffer containing 1 M NaCl than in 0.1 M NaCl. However, this point will not be finally settled until homogenous, high mol. wt. DNA ligase I becomes available. The first report on a DNA ligase in mammalian cells [23] dealt with the properties of the low mol. wt. form of DNA ligase I.

Mammalian DNA ligase I acts by the same general mechanism as the microbial enzymes, with the intermediate formation of ligase–adenylate and DNA–adenylate complexes [1–5,34]. Thus, the mammalian enzyme catalyzes an ATP-dependent pyrophosphate exchange reaction [23], and a covalent enzyme–adenylate complex has been isolated after incubation of purified DNA ligase I with radioactive ATP [25]. This ligase–adenylate complex can join single strand

breaks in DNA in the absence of ATP, and one adenylate residue is released in each repair event. When DNA ligase I is purified by standard methods from calf thymus, part of the enzyme is recovered in the form of such an enzyme–AMP complex, and a small amount of ATP-independent DNA ligase activity can consequently be observed with the enzyme preparations [25]. Similar results have been obtained with a DNA ligase in the chromatin fraction of rabbit bone marrow [35] and with a rat liver cytoplasm DNA ligase [36]. A covalent DNA–AMP complex that is, presumably, a second reaction intermediate has also been recovered after brief incubation at 0°C of isolated DNA ligase I–AMP complex with DNA containing single strand breaks. Free AMP was released on further incubation of the isolated DNA–AMP complex with DNA ligase I and Mg^{2+}, in agreement with the notion that this complex is a reaction intermediate [26].

The above data indicate that the phage T4 DNA ligase and mammalian DNA ligase I act in the same fashion. A crude cell extract of T4-infected *E. coli* has about 500 times higher DNA ligase activity than the most active mammalian cell extracts under optimal assay conditions [25], and homogeneous T4 DNA ligase is an approx. 2000-fold purified enzyme [37,38]. However, it should not be concluded from such data that it might be necessary to purify the DNA ligase I about 10^6 times in order to obtain a homogenous enzyme. Instead, the molecular activity of this mammalian enzyme appears to be much lower than that of T4 ligase. From the amount of active ligase–AMP complex formed, it may be estimated that the 1000-fold purified calf thymus DNA ligase I [15] is at least 5% pure, but that the molecular activity of the enzyme appears to be at least 50 times lower than that of the T4 enzyme. The homogenous *E. coli* DNA ligase [34] also has much lower activity than the T4 enzyme.

Mammalian DNA ligase I joins single strand breaks in DNA with 5′-phosphate and 3′-hydroxy termini independent of the base composition of the DNA [28] In addition to the joining of single strand breaks in DNA, it has been observed with the phage T4 DNA ligase that it can also use a polyribonucleotide chain as a template for the joining of polydeoxyribonucleotides while the *E. coli* DNA ligase can not perform the latter reaction [39–41]. Mammalian DNA ligase I is unable to join hybrid substrates of the type poly(rA) · oligo(dT), and clearly differs from the ATP-dependent T4 ligase in this regard. This restricted substrate specificity was first found for the human DNA ligase I [28] and has been confirmed with the calf thymus enzyme [31].

Actively growing mammalian cells contain more DNA ligase activity than non-growing cells. Thus, the total DNA ligase activity in extracts from regenerating rat liver is about five times higher than in extracts from normal liver [42–44,36]. Similarly, phytohemagglutinin-activated human lymphocytes have more DNA ligase activity than untreated lymphocytes [45], and growing liver or kidney tumors contain more DNA ligase than normal livers or kidneys [44,46]. In a recent study of rat liver regeneration in which DNA ligase I and II activities were separately measured, it was found that the increased ligase activity in the regenerating tissue was due to a large increase in DNA ligase I activity, while DNA ligase II levels did not change significantly [19]. It has also been observed that DNA ligase I, in contrast to DNA ligase II, is most abundant in tissues with a high proportion of dividing cells [15]. These data indirectly suggest that DNA ligase I is active in DNA replication, possibly acting together with the mammalian DNA polymerases α and/or γ, which show similar responses to cell proliferation [6–11].

4. Mammalian DNA ligase II

DNA ligase II was discovered as an enzyme with chromatographic properties different from DNA ligase I, and hydroxyapatite chromatography remains a convenient method for separating the enzymes [14,15]. DNA ligase II only accounts for 5–20% of the total DNA ligase activity in actively growing mammalian cells and is more labile than DNA ligase I. Consequently, it has so far not been studied to the same extent as DNA ligase I, and the reaction mechanism of the enzyme has not been established. DNA ligase II is not antigenically related to DNA ligase I. Thus, rabbit ligase I antibodies do not detectably inhibit DNA ligase II, nor do they interact with the enzyme to form a complex precipitable with goat antibodies against rabbit IgG [15]. Further, the level of DNA ligase II does not appear to vary significantly between non-growing and growing cells,

in contrast to that of DNA ligase I. This means that in normal rat livers, DNA ligase II accounts for about 80% of the total DNA ligase activity, while in regenerating rat livers, which have much higher total DNA ligase activity, the DNA ligase II only accounts for 20–25% of the total activity [19].

DNA ligase II has been partly purified and characterized from calf thymus [14,15], rat liver [16,18,19], and baby hamster kidney cells [17]. The enzyme from rat liver nuclei is 7000-fold purified in comparison with a liver extract but not homogenous [18]. DNA ligase II has a pH optimum at pH 7.8, but in contrast to DNA ligase I it shows very little activity below pH 7.0. It requires Mg^{2+} or Mn^{2+} for activity, with an optimal concentration at about 0.01 M Mg^{2+}, and Ca^{2+} does not satisfy this requirement. At suboptimal concentrations of Mg^{2+} ions, the enzyme activity is stimulated by the presence of NH_4^+ or similar monovalent ions. Neutral salts at higher concentrations are inhibitory, but DNA ligase II from rat or hamster is less sensitive in this regard than either DNA ligase I from the same sources or DNA ligase II from calf tissues. Thus, rat DNA ligase II is actually stimulated two-fold by the presence of 0.14 M NaCl in standard reaction mixtures and shows 30% remaining activity even at 0.30 M NaCl, while calf DNA ligase II is 50% inhibited by 0.10 M NaCl. The enzyme contains essential sulfhydryl groups, as a reducing agent is required for optimal activity and *N*-ethylmaleimide is strongly inhibitory. DNA ligase II requires ATP as a cofactor, and several other nucleoside triphosphates or NAD^+ can not satisfy this cofactor requirement. However, the affinity for ATP is low, as the K_M for ATP is $4 \times 10^{-5} - 1 \times 10^{-4}$ M, a 100-fold higher value than that observed with DNA ligase I [18,31]. DNA ligase II is not inhibited by 10^{-2} M caffeine or by 10^{-3} M hydroxyurea [31]. The enzyme is unusually heat-labile and is inactivated with a half-life of 5 min at 42°C when heated under the conditions described above for DNA ligase I. DNA ligase II has a mol. wt. of about 85 000 and an asymmetric conformation [14]. Estimates of the mol. wt. of the enzyme from gel filtration experiments [16,18] are therefore slightly too high. In addition to joining single-strand breaks in double-stranded DNA and polydeoxynucleotides independent of base composition [18], DNA ligase II can also join oligo(dT) chains hydrogen-bonded to poly(rA) at 5–10% of the rate of joining poly(dA) · oligo(dT), and thus appears to have a broader substrate specificity than DNA ligase I [31].

5. Mitochondrial DNA ligase

Mitochondria contain DNA as well as several enzymes of DNA metabolism. The mitochondrial DNA polymerase has different properties from the DNA polymerases α, β, and γ, and is a fourth distinct DNA polymerase of mammalian cells [6–11]. On the other hand, all enzymes associated with mitochondria are not necessarily unique to that cellular compartment, e.g. the mitochondrial thymidine kinase may have identical properties with one of two extramitochondrial thymidine kinases, so the same enzyme appears to be found at two subcellular locations in that case [47]. Mitochondria contain little DNA ligase activity, and neither DNA ligase I nor ligase II is a predominantly mitochondrial enzyme [15]. Recently, a DNA ligase has been isolated from rat liver mitochondria [20]. The activity is released by treatment with hypotonic solutions or neutral detergents as expected for an intra-mitochondrial enzyme, and it is very unstable unless a protease inhibitor is present during the initial cell homogenization. This DNA ligase appears to be identical with rat DNA ligase II, while it is clearly different from DNA ligase I. Thus, the mitochondrial enzyme has a mol. wt. of about 100 000 and a high K_M for ATP, and the activity is stimulated by the presence of 0.15 M NaCl in the reaction mixtures. Further, the mitochondrial enzyme behaves like DNA ligase II during gradient chromatography on phosphocellulose, and both activities have the same pH dependence and optimal $MgCl_2$ concentration [20]. It would appear that the same enzyme is present at two subcellular locations, i.e. in nuclei and in mitochondria.

6. Virus-induced DNA ligases

By analogy with the phage-induced DNA ligases in *E. coli*, it seems likely that large animal viruses, which are known to induce several different enzymes of DNA metabolism, would also code for a DNA ligase. However, relatively little work has been done

on this subject. In vaccinia virus-infected HeLa cells, the DNA ligase activity of the cytoplasm fraction increases 13-fold 3 h after infection [48]. The subcellular location and the induction kinetics, which were similar to those of other early enzymes believed to be virus-coded, suggest that an ATP-dependent DNA ligase is induced by vaccinia virus, but the enzyme has not been purified or further characterized. No DNA ligase activity was found in purified vaccinia virus particles treated with 2-mercaptoethanol and Nonidet P-40 to activate the particle-associated RNA polymerase [48]. In nuclear extracts of baby hamster kidney cells infected with *Herpes simplex*, an ATP-dependent DNA ligase activity has been found that does not adsorb to DEAE-cellulose under conditions when all detectable DNA ligase activity of uninfected cells is retained [49]. These data suggest that a new DNA ligase may be induced by the virus, or alternatively that a host cell ligase in infected cells shows altered chromatographic properties. Many different temperature-sensitive mutants of *H. simplex* have recently been isolated [50], so it might be possible to obtain direct evidence for the existence of a virus-coded DNA ligase in this case by searching for a virus mutant inducing a defective ligase.

It seems less likely that small animal viruses induce their own DNA ligases, and for small, well characterized viruses such as papova viruses or RNA tumor viruses this notion can now essentially be ruled out, as the gene products of these viruses have been extensively studied. A two-fold increase in ATP-dependent DNA ligase activity is found after infection of resting mouse cells with polyoma virus, but this quite probably only reflects an increase in host enzyme activity in response to increased cellular DNA synthesis after virus infection [29,48]. Moreover, in human embryonic kidney cells infected with adenovirus 2 or 12 there is no increase in DNA ligase activity or any other indications of a virus-induced ligase [51]. Virions of RNA tumor viruses contain several enzymes of DNA metabolism, but with the exception of the virus-coded RNA-directed DNA polymerase these are likely to be host enzymes. The DNA ligase activity detected in disrupted virions of Rous sarcoma virus first appeared unusual in that no cofactor requirement could be observed [52]. However, it was subsequently found that the virions contained ATP and other nucleotides [53]. It may be noted that DNA ligase I functions well even at low ATP concentrations, so if this enzyme is trapped in virions, the small amounts of ATP intrinsically present may well have sufficed to support a reaction. Purified cores of Rous sarcoma virus have higher specific ligase activity than disrupted virions, indicating that the enzyme is associated with the cores [53].

7. DNA ligases in other eukaryotes

DNA ligases from plant cells have been found and partly characterized in three different laboratories [54–56]. A DNA ligase has been purified 600-fold from pea roots [54], and ligase activity has also been observed in extracts of cucumber, spinach, soybean [54], and carrot cells [55], as well as in microsporocyte cells from lilies [56]. In all cases, the plant DNA ligase required Mg^{2+} and ATP, and no activity remained when ATP was replaced with NAD^+ or GTP. Further, a sulfhydryl compound was necessary for optimum activity, and the levels of DNA ligase activity in cell extracts were similar to those found with mammalian tissues. It is not known if there is more than one DNA ligase present in plant cells, and no studies on the reaction mechanism of a plant ligase have been performed. Extracts of plant roots contain two to three times as much DNA ligase activity as extracts of leaves, and most of this ligase is found in the cell nuclei. Chloroplasts from pea leaves contained about 22% of the total DNA ligase activity of leaves, while little ligase activity was detected in isolated mitochondria [54]. The finding of substantial amounts of ATP-dependent DNA ligase activity in chloroplasts is interesting, as these organelles contain covalently closed circular DNA [57]. In other experiments, it has been observed that the amount of DNA ligase activity in crude cell extracts from naturally synchronous lily microspores varies considerably during the cell cycle, but it seems difficult to interpret these changes [56,58,59]. No detailed studies have to our knowledge been performed on DNA ligases in lower eukaryotes. However, an ATP-dependant DNA ligase has been partly purified from yeast (O. Berglund, personal communication).

8. Other ligase activities in mammalian cells

Two different ligases are induced in *E. coli* by

phage T4, the wellknown DNA ligase [1–5] and an ATP-dependent RNA ligase [60]. The latter enzyme converts single-stranded poly(rA) to a circular form, and extends the chain length of a poly(rI)·poly(rC) substrate [60,61]. It has recently been reported that this enzyme also can join DNA at a slow rate, suggesting a role in DNA metabolism for the enzyme [62], but its physiological function is presently unknown. An RNA ligase activity has been reported to be present in the cytoplasm fraction of mammalian cells [61,63], but the experiments could not be confirmed in a separate investigation [64]. Neither DNA ligase I nor DNA ligase II joined poly(rI)·poly(rC) to a detectable extent under conditions when the T4 RNA ligase was clearly active, so neither of these mammalian DNA ligases seems to be able to act as an RNA ligase [65].

In addition to DNA ligase, all cells apparently contain an enzyme that can untwist DNA by introducing a single-strand break and then rejoin it [66]. Such a 'nicking–closing' enzyme has been purified from mammalian cell nuclei [67–69]. This enzyme has no cofactor requirement, and it has so far not been possible to dissociate the 'nicking' activity from the joining activity. Thus, it is clearly a different enzyme from the mammalian DNA ligases discussed previously in this review.

9. Physiological aspects

The presence of two different DNA ligases in mammalian cells makes it seem likely that these two enzymes play different roles in mammalian DNA metabolism, and it would be of obvious interest to define their physiological functions. Several methods are now available to characterize the in vivo properties of mammalian enzymes acting on DNA, and preliminary results have already been obtained by three different approaches. Conditionally lethal mutant cell lines with defective DNA synthesis have been isolated from mouse cell lines [70,71]. One such mutant, *ts* A1S9, shows a defect in joining of newly synthesized DNA fragments to longer chains while DNA repair ability remains normal under the restrictive conditions. It was proposed that *ts* A1S9 cells might have a defect in a DNA ligase specifically involved in DNA replication [70]. However, direct measurements of the DNA ligase I and II levels in this cell line have not yet been performed. In another type of experiment, the total DNA ligase activity in extracts from DNA repair-defective human fibroblasts derived from different complementation groups of xeroderma pigmentosum was measured. No clear difference between the xeroderma pigmentosum cells and control fibroblasts could be discerned [72], but it should be noted that DNA ligase I and DNA ligase II were not separately measured. DNA-synthesizing semi-in vitro systems have recently been established from several types of uninfected and virus-infected mammalian cells. It has been shown that isolated nuclei from polyoma virus-infected mouse cells continue to replicate virus DNA with the aid of cellular nuclear enzymes, but that during extensive washing of the nuclei cellular enzymes leak out so that DNA synthesis is reduced. Addition of 1000-fold purified mammalian DNA ligase I to such enzyme-depleted nuclei results in an increase in the chain length of the newly synthesized DNA, showing that DNA ligase I can serve to join nascent fragments of DNA in this semi-in vitro system [73]. It seems likely that this type of complementation test could also be applied to mutant cells such as the *ts* A1S9 line. A detailed delineation of the physiological roles of the two mammalian DNA ligases and other mammalian enzymes of DNA metabolism, employing the techniques of molecular genetics so successfully used with micro-organisms, no longer appears insurmountably difficult.

References

[1] Richardson, C. C. (1969) Ann. Rev. Biochem. 38, 795–840.
[2] Gellert, M. (1971) in: Procedures in Nucleic Acid Research, Vol. 2, pp. 875–888 (Cantoni, G. L. and Davies, D. R., eds.) Harper and Row, New York.
[3] Lehman, I. R. (1974) in: The Enzymes, 3rd Edn., Vol. 10, pp. 237–259 (Boyer, P. D., ed.) Academic Press, New York.
[4] Lehman, I. R. (1974) Science 186, 790–797.
[5] Gaziev, A. I. (1974) Advances of Modern Biology (Moscow) 78, 171–187.
[6] Kornberg, A. (1974) DNA Synthesis, pp. 152–172 W. H. Freeman Co., San Francisco.
[7] Fansler, B. F. (1974) Int. Rev. Cytol., Suppl. 4, pp. 363–415 Academic Press, New York.

[8] Loeb, L. A. (1974) in: The Enzymes, 3rd Edn., Vol. 10, pp. 173–209 (Boyer, P. D., ed.) Academic Press, New York.
[9] Bollum, F. J. (1975) Progr. Nucleic Acid Res. and Mol. Biol. 15, 109–144 (Cohn, W. E., ed.) Academic Press, New York.
[10] Weissbach, A. (1975) Cell 5, 101–108.
[11] Holmes, A. M. and Johnston, I. R. (1975) FEBS Lett. 60, 233–243.
[12] Laipis, P. J., Olivera, B. M. and Ganesan, A. T. (1969) Proc. Nat. Acad. Sci. USA 62, 289–296.
[13] Becker, A., Lyn, G., Gefter, M. and Hurwitz, J. (1967) Proc. Nat. Acad. Sci. USA 58, 1996–2003.
[14] Söderhäll, S. and Lindahl, T. (1973) Biochem. Biophys. Res. Commun. 53, 910–916.
[15] Söderhäll, S. and Lindahl, T. (1975) J. Biol. Chem. 250, 8438–8444.
[16] Teraoka, H., Shimoyachi, M. and Tsukada, K. (1975) FEBS Lett. 54, 217–220.
[17] Evans, D. G., Ton, S. H. and Keir, H. M. (1975) Biochem. Soc. Trans. 3, 1131–1132.
[18] Zimmerman, S. B. and Levin, C. J. (1975) J. Biol. Chem. 250, 149–155.
[19] Söderhäll, S. (1976) Nature 260, 640–642.
[20] Levin, C. J. and Zimmerman, S. B. (1976) Biochem. Biophys. Res. Commun. 69, 514–520.
[21] Lynch, W. E., Surrey, S. and Lieberman, I. (1975) J. Biol. Chem. 250, 8179–8183.
[22] Herrick, G., Spear, B. B. and Veomett, G. (1976) Proc. Nat. Acad. Sci. USA 73, 1136–1139.
[23] Lindahl, T. and Edelman, G. M. (1968) Proc. Nat. Acad. Sci. USA 61, 680–687.
[24] Bertazzoni, U., Mathelet, M. and Campagnari, F. (1972) Biochim. Biophys. Acta 287, 404–414.
[25] Söderhäll, S. and Lindahl, T. (1973) J. Biol. Chem. 248, 672–675.
[26] Söderhäll, S. (1975) Eur. J. Biochem. 51, 129–136.
[27] Spadari, S., Ciarrocchi, G. and Falaschi, A. (1971) Eur. J. Biochem. 22, 75–78.
[28] Pedrali-Noy, G. C. F., Spadari, S., Ciarrochi, G., Pedrini, A. M. and Falaschi, A. (1973) Eur. J. Biochem. 39, 343–351.
[29] Beard, P. (1972) Biochim. Biophys. Acta 269, 385–396.
[30] Young, H., Ton, S. H., Morrice, L. A. F., Feldberg, R. S. and Keir, H. M. (1973) Biochem. Soc. Trans. 1, 520–522.
[31] Söderhäll, S. and Lindahl, T., unpublished data.
[32] Ray, R. K., Reuber, R., Molineux, I. and Gefter, M. (1974) J. Biol. Chem. 249, 5379–5381.
[33] Barry, J. and Alberts, B. (1972) Proc. Nat. Acad. Sci. USA 69, 2717–2721.
[34] Modrich, P. and Lehman, I. R. (1973) J. Biol. Chem. 248, 7502–7511.
[35] Gaziev, A. I. and Kuzin, A. (1973) Eur. J. Biochem. 37, 7–11.
[36] Austin, G. E. (1975) Fed. Proc. 34, 701.
[37] Weiss, B., Jacquemin-Sablon, A., Live, T. R., Fareed, G. C. and Richardson, C. C. (1968) J. Biol. Chem. 243, 4543–4555.
[38] Panet, A., van de Sande, J. H., Loewen, P. C., Khorana, H. G., Raae, A. J., Lillehaug, J. R. and Kleppe, K. (1973) Biochemistry 12, 5045–5050.
[39] Kleppe, K., van de Sande, J. H. and Khorana, H. G. (1970) Proc. Nat. Acad. Sci. USA 67, 68–73.
[40] Fareed, G. C., Wilt, E. M. and Richardson, C. C. (1971) J. Biol. Chem. 246, 925–932.
[41] Nath, K. and Hurwitz, J. (1974) J. Biol. Chem. 249, 3680–3688.
[42] Tsukada, K. and Ichimura, M. (1971) Biochem. Biophys. Res. Commun. 42, 1156–1161.
[43] Tsukada, K. (1974) Biochem. Biophys. Res. Commun. 57, 758–762.
[44] Miyaki, M. and Ono, T. (1972) GANN Jap. J. Cancer Res. 63, 251–260.
[45] Pedrini, A. M., Nuzzo, F., Ciarrocchi, G., Dalpra, L. and Falaschi, A. (1972) Biochem. Biophys. Res. Commun. 47, 1221–1226.
[46] Tsukada, K., Hokari, S., Hayasaki, N. and Ito, N. (1972) Cancer Res. 32, 886–888.
[47] Nawata, H. and Kamiya, T. (1975) J. Biochem. 78, 1215–1224.
[48] Sambrook, J. and Shatkin, A. J. (1969) J. Virol. 4, 719–726.
[49] Evans, D. G. and Keir, H. M. (1975) Biochem. Soc. Trans. 3, 1260–1262.
[50] Hay, J., Moss, H., Jamieson, A. T. and Timbury, M. C. (1976) J. Gen. Virol. 31, 65–73.
[51] Ledinko, N. (1970) Proc. Soc. Exp. Biol. Med. 133, 707–710.
[52] Mizutani, S., Temin, H. M., Kodama, M. and Wells, R. T. (1971) Nature New Biol. 230, 232–235.
[53] Mizutani, S. and Temin, H. M. (1971) J. Virol. 8, 409–416.
[54] Kessler, B. (1971) Biochim. Biophys. Acta 240, 496–505.
[55] Tsukada, K. and Nishi, A. (1971) J. Biochem. 70, 541–542.
[56] Howell, S. H. and Stern, H. (1971) J. Mol. Biol. 55, 357–378.
[57] Kolodner, R. and Tewari, K. K. (1972) J. Biol. Chem. 247, 6355–6364.
[58] Howell, S. H. and Hecht, N. B. (1971) Biochim. Biophys. Acta 240, 343–352.
[59] Stern, H. and Hotta, Y. (1973) Ann. Rev. Genet. 7, 37–66.
[60] Silber, R., Malathi, V. G. and Hurwitz, J. (1972) Proc. Nat. Acad. Sci. USA 69, 3009–3013.
[61] Linné, T., Oberg, B. and Philipson, L. (1974) Eur. J. Biochem. 42, 157–165.
[62] Snopek, T. J., Sugino, A., Agarwal, K. L. and Cozzarelli, N. R. (1976) Biochem. Biophys. Res. Commun. 68, 417–424.

[63] Cranston, J. W., Silber, R., Malathi, V. G. and Hurwitz, J. (1974) J. Biol. Chem. 249, 7447–7456.
[64] Bedows, E., Wachsman, J. T. and Gumport, R. I. (1975) Biochem. Biophys. Res. Commun. 67, 1100–1107.
[65] Linné, T., personal communication.
[66] Wang, J. C. (1971) J. Mol. Biol. 55, 523–533.
[67] Champoux, J. J. and Dulbecco, R. (1972) Proc. Nat. Acad. Sci. USA 69, 143–146.
[68] Keller, W. (1975) Proc. Nat. Acad. Sci. USA 72, 2550–2554.
[69] Vosberg, H. P. and Vinograd, J. (1976) Biochem. Biophys. Res. Commun. 68, 456–464.
[70] Sheinin, R. (1976) Cell 7, 49–57.
[71] Slater, M. L. and Ozer, H. L. (1976) Cell 7, 289–295.
[72] Pedrini, A. M., Dalpra, L., Ciarrocchi, G., Pedrali-Noy, F., Spadari, S., Nuzzo, F. and Falaschi, A. (1974) Nucleic Acids Res. 1, 193–202.
[73] Otto, B. and Reichard, P. (1975) J. Virol. 15, 259–267.