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MAMMALIAN DNA LIGASES

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PERSPECTIVES

Recent successful demonstrations of DNA replication (1), excision repair (2), and recombinational repair (3) in soluble extracts of mammalian cells have stimulated research aimed at a thorough characterization of the enzymes that catalyze these processes. The ultimate goal of such biochemical investigations

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is to correctly reproduce, with purified proteins, the events and pathways that allow the regulated synthesis and maintenance of cellular DNA. The general approach is the same as that initially employed for characterization of DNA replication functions in *Escherichia coli* and its bacteriophages, that is, identification, purification, and characterization of relevant proteins by in vitro complementation assays. In addition to the discovery of novel mammalian factors required for DNA replication and repair, such projects have stimulated further investigation of the roles of those enzymes previously known to be active in DNA synthesis.

The DNA ligases are a case in point. After the discovery of this enzyme activity in the mid-1960s and an intense research effort in several laboratories over the next 5–10 years, the basic reaction mechanism and functions of microbial DNA ligases were clarified. General interest then waned and, in spite of the widespread use of DNA ligases as essential reagents in recombinant DNA research, very little biochemical work on their properties appeared. In the past few years the situation has changed. The identification of several distinct DNA ligases in mammalian cell nuclei, and the occurrence of DNA-joining defects in certain individuals with both clinical symptoms of immune deficiencies and cellular hypersensitivity to DNA-damaging agents, have encouraged detailed studies of DNA ligases. These projects can now be extended by construction of transgenic mouse models of the relevant human disorders.

Two potentially fruitful research topics are still in their infancies. Firstly, the biochemical investigation of DNA ligases in the genetically well-characterized budding and fission yeasts, which should provide excellent model systems for functional assignments, remains to be performed. Secondly, future identification of specific chemical inhibitors of the different mammalian DNA ligases should help to clarify the relative roles of these enzymes. Studies with inhibitors, although admittedly only yielding somewhat preliminary and tentative results, nevertheless have been very important for the assignments of specific cellular functions to different DNA polymerases, RNA polymerases, and DNA topoisomerases, as well as for manipulation of their activities. If, for example, different DNA ligases were responsible for DNA joining during homologous recombination than during illicit recombination, selective inhibition of the latter process would obviously be helpful in gene correction experiments. Furthermore, as is already the case for DNA topoisomerases, specific enzyme inhibitors that prevent DNA sealing may turn out to be clinically useful as cytotoxic drugs.

REACTION MECHANISM

The mechanism for joining of DNA strand interruptions by DNA ligases has been elucidated (4) and is described in standard textbooks of biochemistry.

The reaction is initiated by the formation of a covalent enzyme-adenylate complex. Mammalian and viral DNA ligases employ ATP as cofactor, whereas bacterial DNA ligases use NAD to generate the adenylyl group. The ATP is cleaved to AMP and pyrophosphate with the adenylyl residue linked by a phosphoramidate bond to the ϵ-amino group of a specific lysine residue at the active site of the protein (5, 6). Several enzymes that interact with nucleotide substrates, including DNA ligases, contain an unusually reactive lysine residue in their active site, which can form a Schiff base with pyridoxal phosphate. In consequence, the activity of mammalian DNA ligase I (and presumably other DNA ligases) is inhibited in vitro by pyridoxal phosphate (6). Detection of the enzyme-adenylate complex as a radioactively labelled intermediate by SDS-polyacrylamide gel electrophoresis and autoradiography provides a convenient method for the assay of DNA ligases in partially purified protein fractions. ATP-dependent DNA ligases can employ analogues such as dATP, but the anomalous complexes formed with some ligases may function poorly in subsequent steps of the DNA-joining reaction (7). The activated AMP residue of the DNA ligase–adenylate intermediate is transferred to the 5' phosphate terminus of a single strand break in double-stranded DNA to generate a covalent DNA-AMP complex with a 5'–5' phosphoanhydride bond. This reaction intermediate has also been isolated for microbial and mammalian DNA ligases, but is more short-lived than the adenylylated enzyme. In the final step of DNA ligation, unadenylylated DNA ligase is required for the generation of a phosphodiester bond and catalyzes displacement of the AMP residue through attack by the adjacent 3'-hydroxyl group on the adenylylated site.

Two related enzymes also employ this reaction mechanism of enzyme-adenylate formation, followed by transfer of the AMP residue to activate a 5'-phosphate residue of a nucleic acid substrate prior to joining. One of these is bacteriophage T4 RNA ligase, the product of T4 gene 63, which can catalyze the ligation of single-stranded ribo-oligonucleotides and deoxyribo-oligonucleotides with 5'-terminal phosphates and 3'-terminal hydroxyl groups (8). In contrast to DNA ligases, RNA ligases show no requirement for a complementary template strand in the nucleic acid substrate. The other enzyme is yeast tRNA ligase, an enzyme required for splicing of tRNA precursors. The locations of the adenylylated lysine residue within the sequences of the T4 RNA ligase and yeast tRNA ligase have been defined (9, 10). Recently, the active site for enzyme-adenylate formation of mammalian DNA ligase I was located by isolation and sequencing of a radioactively labelled tryptic peptide-AMP complex from bovine DNA ligase I, and by identification of the same peptide within the predicted reading frame of the human cDNA encoding this enzyme (6). The tryptic peptide contained a single internal lysine residue, protected against proteolysis by adenylylation. Since several DNA ligase genes have been cloned and sequenced, it is

possible to predict the locations of the active site regions in all these enzymes by partial peptide sequence homology with the mammalian enzyme (Figure 1). The distance between the postulated adenylylation site and the carboxyl terminus of these enzymes is also practically invariant between the ATP-dependent DNA ligases.

The amino acid sequences of human DNA ligase I and the DNA ligase of the fission yeast, *Schizosaccharomyces pombe*, are virtually identical in the region of the active site, although the two enzymes show only 44% overall identity (11, 12). By comparison with DNA ligases from more distantly related organisms, several strongly conserved residues may be identified. The active lysine residue in DNA ligases is bracketed by a hydrophobic amino acid residue on each side, and the sequence E-KYDG-R is common to enzymes from such different sources as mammalian cells, yeasts, vaccinia virus, and bacteriophage T7. The T4 RNA ligase shows so little homology with DNA ligases that its sequence could not be used to predict the active sites in DNA ligases (9), but even in this case certain features are retained, such as the conserved glycine residue at position +3.

The mechanistic analogy between DNA ligases and RNA ligases has been confirmed and extended by recent site-specific mutagenesis experiments with T4 RNA ligase (9) and human DNA ligase I (13). As expected, replacement of the AMP-binding reactive lysine at the active site by another amino acid causes total loss of enzyme activity. In DNA ligase I, substitutions of the

		-7	-6	-5	-4	-3	-2	-1		+1	+2	+3	+4	+5	+6	+7	+8	+9	
Human	561	A	A	F	T	C	E	Y	K	Y	D	G	Q	R	A	Q	I	H	577
Sch.pombe	409	A	A	F	T	C	E	Y	K	Y	D	G	E	R	A	Q	V	H	425
S.cerevisiae	412	E	T	F	T	S	E	Y	K	Y	D	G	E	R	A	Q	V	H	428
Vaccinia	224	S	G	M	F	A	E	V	K	Y	D	G	E	R	V	Q	V	H	240
T7	27	G	Y	L	I	A	E	I	K	Y	D	G	V	R	G	N	I	C	43
T3	27	G	Y	L	I	A	D	C	K	Y	D	G	V	R	G	N	I	V	43
T4	152	F	P	A	F	A	Q	L	K	A	D	G	A	R	C	F	A	E	168
E.coli	108	V	T	W	C	C	E	L	K	L	D	G	L	A	V	S	I	L	124
T.thermophilus	111	F	A	Y	T	V	E	H	K	V	D	G	L	S	V	N	L	Y	127
T4 RNA ligase	92	D	V	D	Y	I	L	T	K	E	D	G	S	L	V	S	T	Y	108
tRNA ligase	107	G	P	Y	D	V	T	I	K	A	N	G	C	I	I	F	I	S	123

Figure 1 Active site regions for enzyme-adenylate formation in DNA and RNA ligases. Sequences are aligned by homology with the active site peptide of mammalian DNA ligase I. The reactive lysine residue within the mammalian enzyme and the RNA ligases, and that predicted in the other proteins, is shown in outline. The peptide sequences for the *Thermus thermophilus* DNA ligase and yeast tRNA ligase are from Lauer et al (142) and Xu et al (10), respectively; references to other sequences are in Tomkinson et al (6).

strongly conserved residues at positions −2, +3, and +5 also lead to very strong reduction or total loss of ability to form an enzyme-adenylate complex. Heaphy et al (9) found that substitution of the Asp residue at +2 in T4 RNA ligase still allowed enzyme-adenylate formation, but that the subsequent joining reaction did not occur. Similar results have been obtained for mammalian DNA ligase I, indicating the existence of shared structural and mechanistic features at the active sites of these two enzymes, although they have no detectable overall sequence homology. An antibody directed against the active site peptide of mammalian DNA ligase I does not bind to or inhibit the native enzyme (A. E. Tomkinson, T. Lindahl, unpublished data), indicating that this relatively hydrophobic sequence is protected within a groove or cleft of the protein.

DNA LIGASE I

Structure

Peptide sequences from the homogeneous bovine enzyme were used to synthesize corresponding oligonucleotide probes, and a partial cDNA sequence was isolated from a human cDNA library. A full-length human cDNA encoding DNA ligase I was subsequently obtained by functional complementation of a *Saccharomyces cerevisiae cdc9* temperature-sensitive DNA ligase mutant (11). The full-length cDNA encodes a 102-kDa protein of 919 amino acid residues. There is no marked sequence homology to other known proteins except for microbial DNA ligases. The active site lysine residue is located at position 568. The intact enzyme has been purified to apparent homogeneity from calf thymus (14, 15), and has also been highly purified from regenerating rat liver (16) and human lymphoblastoid cells (15). DNA ligase I accounts for the majority of the total DNA ligase activity in calf thymus extracts.

There is considerable confusion in the literature about the size of DNA ligase I. This is due to at least four complications:

1. The enzyme migrates anomalously slowly on SDS-polyacrylamide gels, corresponding to a 125–130-kDa protein (14–17). This retardation is largely due to a high proline content in the protein (15), and also to the presence of a small number of phosphoserine residues (18). Expression of the human cDNA encoding DNA ligase I in *S. cerevisiae* results in the production of a protein with the same anomalously slow migration rate during electrophoresis (11).

2. Mammalian DNA ligase I has a markedly asymmetric structure with a frictional ratio of 1.9 (14, 15) and resembles the *E. coli* and T4 DNA ligases in this regard. Consequently, the purified calf thymus enzyme sediments more slowly than expected for a 102-kDa protein on sucrose gradient centrifugation, whereas it elutes earlier than expected on gel filtration. Combination of

the data (19) for the sedimentation coefficient and the Stokes radius determined by gel filtration, nevertheless, allows for determination of an accurate native molecular weight by the Svedberg equation, within 5% of the value determined from the cDNA sequence (15). Thus, estimation of the size of the enzyme by hydrodynamic methods is more accurate than by SDS-polyacrylamide gel electrophoresis. On the other hand, use of gel filtration data by themselves to estimate a molecular mass for a hypothetical globular protein leads to a gross overestimate. This error, rather than enzyme dimerization, explains several reports of 200–240 kDa forms of DNA ligase I in solution.

3. Several antisera against purified DNA ligase I cross-react by immunoblotting with a 200-kDa protein present in crude cell extracts (20, 21). Moreover, a protein of ~200-kDa is one of several proteins in crude cell extracts that can be adenylylated (20, 22). It is not known if these two 200-kDa proteins are the same, and in any case the 200-kDa protein fraction has no DNA ligase activity (22). Nevertheless, it has been proposed by several authors that DNA ligase I might initially be synthesized as a 200-kDa protein that would be proteolytically processed to the active enzyme (17, 20–23). The cDNA sequencing of DNA ligase I and determination of the size of the mRNA (11) invalidate this model. Moreover, a longer cDNA clone has been obtained, which extends the 5' end of the published sequence (11) by 89 basepairs (bp) and places an in-frame stop codon 99 bp upstream of the initial methionine, confirming that this was correctly assigned (23a). We have noted that the 200-kDa protein cross-reacting with antisera against DNA ligase I is readily detectable by immunoblotting if bovine serum albumin is used to block nonspecific protein binding to nitrocellulose filters, but is barely seen if a more effective blocking agent such as nonfat milk is employed; the authentic DNA ligase I signal is not suppressed by either blocking agent (C. Prigent, T. Lindahl, unpublished data). These data indicate that the 200-kDa protein is not directly related to DNA ligase I but could have a common epitope.

4. DNA ligase I is highly sensitive to partial proteolysis, and it is difficult to avoid such degradation during enzyme purification. In calf thymus extracts, and partially purified enzyme fractions, DNA ligase I is attacked slowly by an endogenous protease activity that removes the N-terminal 216 amino acids to generate an active fragment of 703 amino acids. This fragment shows the same DNA-joining activity as the intact enzyme in standard DNA ligase assays, indicating that the N-terminal region of the protein is not required for DNA ligation activity in vitro. The same result is obtained when intact, highly purified DNA ligase I is treated with low concentrations of subtilisin (15) instead of being exposed to proteolysis in cell extracts. The large C-terminal fragment of DNA ligase I is relatively resistant to further proteolysis and apparently represents the catalytic domain of the enzyme.

Furthermore, a truncated human cDNA encoding this domain is able to complement DNA ligase mutants of *S. cerevisiae* (11) and *E. coli* (13). The nonessential N-terminal region contains a large proportion of proline and hydrophilic amino acids, in particular stretches of glutamic acid residues. It shows no detectable homology to the shorter N-terminal regions of the related DNA ligases from *Sch. pombe* and *S. cerevisiae*, although the three proteins are about 50% homologous in their C-terminal regions.

Because of the ready conversion of the intact form of DNA ligase I to an active proteolytic fragment, several studies on DNA ligase I have been concerned with the properties of the active fragment rather than the intact enzyme. This was the case for the first report on a mammalian DNA ligase activity (24) as well as for several more recent investigations (25–29). Other early studies reported on both the intact form and the active fragment of DNA ligase I (30). When mammalian cells growing in tissue culture are lysed instantaneously with hot SDS, followed by immunoblotting analysis with antibodies against DNA ligase I, the intact protein is the only one detected. Thus, the active fragment representing the catalytic domain does not seem to be present in vivo (31).

Deletion of terminal regions from the DNA ligase I cDNA, followed by functional expression in *E. coli*, has provided more precise information on the domain structure of DNA ligase I. The *E. coli* conditional lethal mutant strain SG251, which was originally isolated as a temperature-sensitive DNA replication mutant, and a transductant strain GR501 carrying the same mutation, have a heat-labile NAD-dependent DNA ligase and are unable to join Okazaki fragments at 42 °C (32). The physiological defect can be complemented by the catalytic domain of DNA ligase I, encoded by a fragment of the human cDNA expressed as a lacZ fusion protein (13). A fragment lacking the N-terminal 249 amino acids of DNA ligase I functions well as a DNA ligase in this heterologous in vivo system. This active fragment is 33 amino acids shorter than the catalytic domain generated by proteolysis of DNA ligase I in cell extracts. However, reduction of the size of the domain from 670 to 635 amino acids by a further N-terminal deletion causes complete loss of DNA ligation activity both in vivo and in vitro.

In the C-terminal region of the enzyme, human DNA ligase I shows homology with the DNA ligases of both *Sch. pombe* and *S. cerevisiae*, except that the human and *Sch. pombe* enzymes are 14 amino acids longer at optimal sequence alignment (11). There is little homology between the two proteins in this "tail" sequence (11). This short C-terminal sequence can be removed from human DNA ligase I without functional impairment, but deletion of 8 additional residues at the C-terminus causes loss of activity (13). The existence of an evolutionarily conserved C-terminal catalytic domain joined to an N-terminal region that is nonessential for enzyme activity in vitro is not

unique to DNA ligase I, but has also been observed for several other mammalian nuclear enzymes acting on DNA including DNA topoisomerase I (33) and the DNA methyltransferase that converts cytosine residues to 5-methylcytosine (34). The role of the N-terminal region of DNA ligase I remains unclear, although it contains sequences that could function as nuclear translocation signals and stretches of acidic residues that might mediate interactions with chromatin or the nuclear scaffold. The N-terminal region might also be employed in species-specific protein-protein interactions during DNA replication. A cDNA subclone expressing this fragment in vivo might have a dominant negative effect and could elucidate the function of this hydrophilic region of the enzyme.

Gene Structure and Chromosome Mapping

The cloning of the human DNA ligase I cDNA allows for an analysis of the gene encoding this enzyme (11, 35). Southern hybridization analysis of EcoRI-digested genomic DNA with the cDNA probe identified a discrete number of hybridizing bands and gave no indication for the existence of multiple related genes or pseudogenes. The size of the gene is estimated to be 45–50 kilobases (kb), and sequence analysis of genomic clones has shown that the gene contains 27 introns (23a). A single mRNA of 3.2 kb is transcribed from the gene.

DNA hybridization with material from rodent-human somatic cell hybrids retaining subsets of human chromosomes showed that the DNA ligase I gene is located on chromosome 19. Four overlapping positive cosmids forming a contig have been identified in an arrayed human chromosome 19–specific cosmid library (35). More detailed mapping by in situ hybridization demonstrated that the *LIG1* gene is localized to the long arm of the chromosome, at 19q13.2–13.3. Since three different genes active in DNA repair, *ERCC1* (36), *ERCC2* (37), and *XRCC1* (38), have been mapped previously to this region of chromosome 19, a more detailed study was performed using two-color fluorescence in situ hybridization to position *LIG1* relative to *ERCC1*. The in situ hybridization results, together with genetic and physical data, give an order for the four genes on the long arm of this chromosome of: centromere ——*XRCC1* ——*ERCC2* ——*ERCC1* ——*LIG1* ——-telomere.

A defect in the *XRCC1* gene leads to cellular sensitivity to ethyl methanesulfonate and ionizing radiation, and a reduced capacity to rejoin DNA single-strand breaks (38). The putative *XRCC1* gene product is a 69.5-kDa protein, which is clearly different from DNA ligase I. Furthermore, peptide sequences derived from purified DNA ligase II do not appear in the predicted XRCC1 protein sequence, indicating that the *XRCC1* gene does not encode DNA ligase II. The relationship, if any, of DNA ligase III to XRCC1 remains to be established.

Catalytic Properties

DNA ligase I requires Mg^{2+} and ATP for activity. GTP and NAD are inactive as cofactors. The K_M for ATP is low, 0.5–1 μM (6, 14, 39). This property of the enzyme facilitates assays of the enzyme by measuring DNA ligase–AMP complex formation. The reaction is readily reversed in the presence of pyrophosphate. The active proteolytic fragment of DNA ligase I, which represents the catalytic domain of the enzyme, retains the low K_M for ATP. The DNA-adenylate reaction intermediate in the joining process catalyzed by DNA ligase I has also been isolated and characterized (40). Thus, the general reaction mechanism for this mammalian enzyme appears identical to that established for microbial DNA ligases. DNA ligase I effectively seals single-strand breaks in DNA and joins restriction enzyme DNA fragments with staggered ends. The enzyme is also able to catalyze blunt-end joining of DNA (41). As for other DNA ligases, the latter reaction is stimulated greatly by the macromolecular crowding conditions inflicted by the presence of 10–20% polyethylene glycol in reaction mixtures (42). DNA ligase I is much more effective at blunt-end joining than mammalian DNA ligases II and III (41, 42a), but is less efficient in this regard than T4 DNA ligase. Similarly to microbial DNA ligases and *Drosophila* DNA ligase I (43), mammalian DNA ligase I can act at low efficiency as a topoisomerase, relaxing supercoiled DNA in an AMP-dependent reversal of the last step of the ligation reaction followed by re-ligation (44).

DNA ligase I can join oligo (dT) molecules hydrogen-bonded to poly (dA), but the enzyme differs from T4 DNA ligase in being unable to ligate oligo (dT) with a poly (rA) complementary strand (30, 41). The response with several other polynucleotide-oligonucleotide combinations is variable, but DNA ligase I can join oligo (rA) molecules hydrogen-bonded to poly (dT) (45). The lack of activity with an oligo (dT)·poly (rA) hybrid structure has turned out to be a helpful property of the enzyme, because DNA ligases II and III are able to join this particular substrate. Thus, the latter DNA ligase activities can be specifically followed during enzyme purification even in the presence of an excess of the intact form and active fragments of DNA ligase I (16, 41, 45). However, the sensitivity of the oligo (dT)·poly (rA) substrate to degradation by RNaseH makes this method unsuitable for cell extracts or crude enzyme fractions.

DNA Ligase I is a Phosphoprotein

Labelling of tissue culture cells with ^{32}P orthophosphate followed by immunoprecipitation of DNA ligase I indicated that the enzyme contained radioactive material. Addition of homogeneous calf thymus DNA ligase I to the cell extracts completely suppressed the immunoprecipitation of radioactive material, and established that the cellular DNA ligase I was ^{32}P-labelled.

Approximately one-third of the radioactive material could be released by exposure of the immune complexes to pyrophosphate and was due to the presence of activated enzyme-adenylate reaction intermediates in the cell. However, two-thirds of the radioactive material in DNA ligase I was refractory to release by pyrophosphate but could be converted to an acid-soluble form by treatment with *E. coli* alkaline phosphatase or potato acid phosphatase. Amino acid analysis by gel electrophoresis with appropriate markers demonstrated the presence of phosphoserine, whereas phosphothreonine and phosphotyrosine were not detected (18). Phosphatase treatment of purified calf thymus DNA ligase I caused a small shift in the migration of the enzyme on SDS-polyacrylamide gel electrophoresis. The corresponding molecular mass estimates by this method were 125 kDa for the phosphorylated enzyme isolated from tissue, and 115 kDa for the dephosphorylated enzyme.

Many different cellular proteins are phosphorylated, so these results are by themselves not surprising. However, the phosphorylation of DNA ligase I is of interest because it affects the catalytic activity of the enzyme. Dephosphorylation of DNA ligase I with *E. coli* alkaline phosphatase or potato acid phosphatase caused a drastic reduction in enzyme activity. In the converse experiment, additional phosphorylation of purified calf thymus DNA ligase I with the ubiquitous protein kinase, casein kinase II [CKII; (46)] using either ATP or GTP as cofactor caused an approximately threefold increase in the ability of DNA ligase I to generate the enzyme-adenylate intermediate. Thus, DNA ligase I is activated by protein phosphorylation (18). The reaction is most conveniently followed by using GTP rather than ATP as cofactor for CKII, since this avoids simultaneous adenylylation of the DNA ligase. In this way it was shown that formation and accumulation of the enzyme-AMP complex only occurs with the phosphorylated form of DNA ligase I. Several protein kinases were investigated, including the cAMP-dependent protein kinase A, protein kinase C, and purified p34cdc2 protein kinase, but CKII was the only kinase tested that activated DNA ligase I in vitro. Heparin is a potent inhibitor of CKII and also prevented the activation of DNA ligase I by CKII. In the predicted amino acid sequence of DNA ligase I there are at least three potential phosphorylation sites for CKII. Two of these are located in the N-terminal region of the enzyme outside the catalytic domain, whereas the third occurs within an evolutionarily conserved region just over a hundred amino acid residues from the C-terminus of the enzyme.

Expression of the entire 102-kDa human DNA ligase I in a DNA ligase-deficient *E. coli* strain yields no complementation at 42 °C, whereas a subclone expressing the catalytic domain of the enzyme allows efficient physiological complementation (13). The 102-kDa DNA ligase expressed in *E. coli* did not detectably form the enzyme-adenylate complex on incubation with ATP. However, this form of DNA ligase I was converted to an active

enzyme by pretreatment with CKII and GTP (18). The data indicate that phosphorylation of one or both target serine residues in the N-terminal region of the enzyme is required to avoid suppression of the activity of the catalytic domain. This could provide a mechanism for regulation of DNA ligase I activity during the cell cycle, especially as the long half-life of the protein in vivo (31) would make its regulation at the mRNA level ineffectual.

Subcellular Localization and Functional Properties

Immunofluorescence studies with affinity-purified polyclonal antibodies and monoclonal antibodies against DNA ligase I have established that the enzyme is localized to cell nuclei (31). As also observed for DNA polymerase α (47), DNA ligase I leaches out of cell nuclei during isolation in isotonic sucrose by standard methods. Both DNA polymerase α and DNA ligase I exhibited granular nuclear staining and seem to be excluded from the nucleoli. Such a localization pattern coincides with that of apparent DNA replication complexes (48, 49). Moreover, during Herpes simplex 1 (HSV 1) infection of cells, certain host DNA replication factors are redistributed within nuclei and recruited to viral replication factories, whereas this is not the case for several other nuclear proteins. DNA ligase I is one of the recruited host proteins, as determined by immunofluorescence (50). Since HSV 1, as well as other herpesviruses, does not encode a DNA ligase, it is hardly surprising that the virus employs a host enzyme for replication, and it seems likely that DNA ligase I serves in this function. Adenovirus infection also depends on reprogramming of the host replication machinery, and it is intriguing that the viral regulatory E1A proteins bind to an unidentified host protein of the same size as DNA ligase I (130 kDa by SDS-polyacrylamide gel electrophoresis; 51) as well as modulators of cell growth, such as the retinoblastoma protein (52) and cyclin A (53). However, immunoprecipitation experiments with E1A and DNA ligase I antibodies are inconclusive at this point (C. Prigent and D. Barnes, unpublished). DNA ligase I appears to account for the ligation of Okazaki fragments during SV40 DNA synthesis in a cell-free system (54). In this connection, Malkas et al (55) have isolated a 21S protein complex from HeLa cells, which comprises several replication enzymes and can replicate SV40 DNA in the presence of large T antigen. The enzyme complex contains DNA polymerase α/primase, the DNA polymerase δ–associated factor PCNA, DNA topoisomerase I, an RNaseH, a DNA-dependent ATPase, and DNA ligase I, as well as a number of additional polypeptides (E. F. Baril, personal communication).

The half-life of DNA ligase I in growing tissue-culture cells is about 7 h as determined by pulse-chase experiments (31). The enzyme is present at a much higher level in proliferating than in nongrowing cells, both when measured by activity assays and by immunochemical protein determinations. In contrast,

DNA ligases II and III are present in similar amounts in proliferating and nongrowing cells (14, 16, 56, 57). DNA ligase I is also able to correct the DNA replication defect in conditional lethal DNA ligase mutants of *S. cerevisiae* (11) and *E. coli* (13). Taken together, these results strongly imply a role for DNA ligase I in DNA replication.

Direct evidence for this concept has been obtained recently by the characterization of the human cell line 46BR, described in detail below, as a mutant line with a structural defect in DNA ligase I on the amino acid sequence level (58). This cell line exhibits a strong delay in the rate of joining of Okazaki fragments at 37 °C (59, 60, 60a). Interestingly, not only is the 46BR cell line defective in DNA replication, but the cells are also hypersensitive to several DNA-damaging agents, implying a defect in DNA repair (60–63). These agents include ultraviolet light, simple alkylating agents, and ionizing radiation. The DNA damage induced by these agents is chemically different and is corrected by separate excision-repair processes (64). DNA ligase I apparently completes all these repair pathways, which are largely carried out by different sets of enzymes. In agreement with these data, it has been found that the human DNA ligase I cDNA corrects the defective phenotype of a *S. cerevisiae cdc9* mutant both with regard to replication and several forms of repair (11; L. H. Johnston, personal communication). In conclusion, it would appear that DNA ligase I is the key enzyme for joining Okazaki fragments during lagging-strand DNA synthesis in mammalian cells and also for completion of DNA excision-repair processes.

Activators and Inhibitors

Several reports have appeared on cellular factors that modulate the activity of DNA ligase I. Preliminary observations on a heat-stable protein activator of DNA ligase activity in extracts of the human heteroploid cell line EUE indicated that the stimulatory factor(s) was either very heterogeneous in size or aggregated easily (30, 65). However, the factor could not be replaced with bovine serum albumin and caused a four- to five-fold promotion of DNA ligase I activity. Similarly, the human fibroblast line GM1492, but not several related fibroblast lines, expresses substantial amounts of a heat-stable protease-sensitive factor, which stimulates the activity of calf thymus DNA ligase I (66). The active factor has been partially purified by single-stranded DNA cellulose chromatography.

Induction of differentiation in mouse erythroleukemia cells by growth in the presence of dimethyl sulfoxide causes a fourfold decrease in DNA ligase activity in cell extracts. This reduction in DNA ligase activity may be associated with a decreased replication rate in differentiated cells, as observed in the *Drosophila* and *Xenopus* systems. However, the high levels of heme accumulated in the differentiating erythroleukemia cells may also contribute

to suppress DNA ligase activity (67). In a separate study, a protein inhibitor of DNA ligase I has been extensively purified from HeLa cell extracts (67a). The inhibitor is heat-labile and sensitive to proteases. The specificity of this inhibitor is of interest; it interferes effectively with DNA joining catalyzed by mammalian DNA ligase I but not enzyme-AMP formation, and it has no effect on either mammalian DNA ligase II or T4 DNA ligase. This inhibitor is a protein of about 65 kDa as estimated by gel filtration, and it appears to form a protein-protein complex with DNA ligase I. Thus, the inhibitor initially copurifies with DNA ligase I, but the two proteins can be separated by Mono S ion-exchange chromatography. The purified inhibitor exhibits no detectable phosphatase, ATPase, DNase, or poly ADP-ribose polymerase activity. A modulating effect of poly ADP-ribosylation of DNA ligase II has been reportcd but is controversial (68, 69). Further studies on the significance and binding specificity of the cellular factors that affect DNA ligase activity, and cloning of cDNAs encoding putative protein activators and inhibitors, should clarify their possible roles in modulating DNA ligase activity in vivo.

In a recent survey of ATP analogues, arabinosyl-2-fluoro-ATP was found to inhibit DNA ligase I (69a). Thus, the ligase may be a target in tumor cells treated with the anticancer drug arabinosyl-2-fluoroadenine.

DNA LIGASE II

A DNA ligase distinct from DNA ligase I is more firmly associated with cell nuclei and is only extracted into soluble form by salt-containing buffers (27, 39, 70). The enzyme has been purified from calf thymus as a 68–72-kDa protein of apparent homogeneity, estimated by SDS-polyacrylamide gel electrophoresis, by three different groups (27, 45, 71). The protein has a blocked N-terminal residue. The enzyme is present in this form both in crude cell extracts and in purified form, and there is no detectable proteolytic processing or generation of active fragments during isolation (45, 71). In common with other DNA ligases, this protein exhibits a high frictional ratio, reflecting an apparently asymmetric structure. DNA ligase II is generally found at a lower level of activity than DNA ligase I in mammalian cells and tissues. Moreover, the enzyme is a labile protein, which is rapidly inactivated at 42 °C. This intrinsic lability causes problems during enzyme purification, although some stabilization of the purified active enzyme can be achieved by addition of a neutral detergent such as Tween 20 to 0.2% (45) or a carrier protein such as bovine serum albumin (71). Several groups have documented their inability to detect or purify DNA ligase II. The various reasons for these technical difficulties have been discussed elsewhere (72); possible explanations include irreversible denaturation of the enzyme by exposure to SDS prior to attempted enzyme assays (17), failure to extract the enzyme from nuclei, or loss in the first batch purification step.

The properties of highly purified DNA ligase II initially indicated that the enzyme had been purified to homogeneity from calf thymus. A single narrow protein band was observed on SDS-polyacrylamide gel electrophoresis, and a single amino-terminal sequence was detected by protein sequencing. However, isoelectric focusing followed by SDS-polyacrylamide gel electrophoresis demonstrated the existence of two proteins of slightly different pI present in similar amounts in the preparation (45). Separation of the two proteins by chromato-focusing was inefficient. Sequencing of several peptides from the preparation established that the purified DNA ligase II was heavily contaminated with the abundant cellular structural protein ezrin, also called cytovillin (73, 74). Ezrin/cytovillin contributed the single nonblocked N-terminal sequence of the preparation. DNA ligase II and ezrin/cytovillin are of the same size, and these two proteins also share the unusual property of adsorbing very tenaciously to hydroxylapatite, which consequently is a key purification step for either protein (45, 75, 76). Probably the easiest remedy for this copurification problem is to isolate DNA ligase II from cell nuclei rather than from whole cell extracts (27). The presence of variable amounts of ezrin/cytovillin in DNA ligase II preparations is unlikely to affect the catalytic properties of the DNA ligase, however, and the ezrin/cytovillin may even serve as a carrier protein instead of externally added bovine serum albumin. Since the sequence of ezrin/cytovillin is known, it is easy to deduce which peptides have been derived from this structural protein, and which ones from DNA ligase II, during amino acid sequencing.

The sequence of DNA ligase II appears to be quite different from that of DNA ligase I. Several peptides derived from DNA ligase II (45) show no detectable sequence homology with the predicted coding sequence of the DNA ligase I cDNA (11). The two enzymes show no serological cross-reactivity, that is, neutralizing rabbit antibodies against DNA ligase I do not bind or inhibit DNA ligase II (56), and neutralizing antibodies against DNA ligase II similarly do not inhibit DNA ligase I (71). Furthermore, an antibody directed against a peptide strongly conserved between DNA ligase I, the *Sch. pombe cdc17*$^{+}$-encoded DNA ligase, the *S. cerevisiae CDC9* gene product, and vaccinia virus–encoded DNA ligase (15) readily detects these enzymes by immunoblotting but fails to cross-react with DNA ligase II (45, 77). A recent report of a fingerprinting study indicated that DNA ligase II may have a long sequence around its active site that seems identical to that of DNA ligase I (29), but it is presently unclear whether the peptide analyzed was really derived from the partially purified DNA ligase II or from a contaminating fragment of DNA ligase I. Subtilisin digestion of homogeneous DNA ligase I does not generate a fragment the size of DNA ligase II (15).

DNA ligase II resembles other eukaryotic DNA ligases in requiring ATP as cofactor, but the enzyme differs from DNA ligase I in having a much higher

K_M for ATP. Enzyme preparations from different sources consistently exhibit K_M values of 10^{-5}–10^{-4}M in different laboratories. DNA ligase II forms an enzyme-adenylate complex and apparently acts by the same mechanism as other DNA ligases (45, 71), but complex formation is inefficient and difficult to detect at low ATP concentrations due to the high K_M for the cofactor. The enzyme can catalyze the formation of phosphodiester bonds with an oligo (dT)·poly (rA) substrate, but not with an oligo (rA)·poly (dT) substrate, so it differs completely from DNA ligase I in this regard (41, 45). The cellular function of DNA ligase II remains unknown. Whereas DNA ligase I activity is induced in regenerating rat liver, as expected for an enzyme involved in DNA replication, DNA ligase II levels are unaltered in normal versus regenerating liver (57, 70).

DNA LIGASE III

Two different DNA ligases are present in calf thymus cell extracts that can join the oligo (dT)·poly (rA) substrate refractory to DNA ligase I. The recently detected enzyme, which is larger than DNA ligase II and apparently unrelated to that protein, has been named DNA ligase III (45). An enzyme with the properties of DNA ligase III was partially purified from rat liver cell extracts by Elder & Rossignol (16), who speculated that the activity might represent a more native form of DNA ligase II. However, DNA ligase II is not derived by processing from a larger precursor protein, and partial digestion of purified DNA ligase III with reagent proteases does not generate an active fragment of the size of DNA ligase II (45).

DNA ligase III is a minor DNA ligase activity in calf thymus, and was not detected for a long time because much of the biochemical work on DNA ligases in various laboratories employed this particular source for large-scale enzyme purification. In other cells and tissues, the level of DNA ligase III can approach that of DNA ligase I (58). DNA ligase III elutes before DNA ligase I on gel filtration, and the molecular mass of the active enzyme determined by hydrodynamic methods is about 147 kDa. The most purified fractions of the calf thymus enzyme show only two peptide bands by silver staining after SDS-polyacrylamide gel electrophoresis, corresponding to 100 kDa and 46 kDa (45). The partially purified rat liver enzyme appears to have the same structure (16). The 100-kDa polypeptide contains the AMP residue of a DNA ligase–adenylate complex. These data indicate that DNA ligase III may be a heterodimer comprising two subunits, with the active site for enzyme-AMP formation present in the larger subunit. All other DNA ligases investigated to date have a monomeric structure, although dimerization or aggregation of DNA ligase I has been observed under certain conditions (78, 79). For this reason, it cannot yet be concluded whether the 46-kDa peptide is an essential

and integral part of DNA ligase III, or if it represents a separate protein that binds tenaciously to the 100-kDa DNA ligase component and may be involved in related cellular functions.

DNA ligase III resembles DNA ligase I, and differs from DNA ligase II, in binding only weakly to hydroxylapatite and having a low K_M, $\sim 2\mu M$, for ATP. However, polyclonal antisera against DNA ligase I or the evolutionarily conserved peptide sequence in the C-terminal region of DNA ligase I failed to detect DNA ligase III by immunoblotting (16, 45). These data indicate that DNA ligases I and III are not closely related. DNA ligase III repairs single-strand breaks in DNA efficiently, but is unable to perform either blunt-end joining or AMP-dependent relaxation of supercoiled DNA (42a). The substrate specificity of DNA ligase III differs from that of both DNA ligase I and II in that the enzyme can join both the oligo (dT)·poly (rA) and oligo (rA)·poly (dT) hybrid substrates. However, all three enzymes effectively ligate single-strand breaks with 3'-hydroxyl and 5'-phosphate termini in double-stranded DNA or the synthetic oligo (dT)·poly (dA) substrate (45).

In common with DNA ligase II, DNA ligase III is not induced during rat liver regeneration (16). Furthermore, the presence of the enzyme in the postmicrosomal supernatant of rat liver cells extracted with an isotonic sucrose buffer indicates that the enzyme has a similar subcellular distribution to DNA ligase I, which leaches out of nuclei under such conditions. In contrast, DNA ligase II is not extracted from nuclei with isotonic sucrose (27, 39, 70).

The definition of precise functions for DNA ligases II and III remains a challenging problem. The essential requirements for DNA ligase activity in joining of Okazaki fragments during DNA replication and completion of DNA repair pathways are most likely fulfilled by DNA ligase I. Consequently, it seems probable that DNA ligases II and III are involved in more specialized and less obvious functions. These are at present a matter of speculation. However, there is no shortage of interesting putative roles for the enzymes. For example, which DNA ligase joins larger discrete DNA replication intermediates (80), or adjacent replicons? Which enzyme is responsible for the joining of unusual replication intermediates and DNA structures occurring at telomeres (81) and perhaps at centromeres? The presence of stretches of single-stranded DNA up to 10^5 nucleotides long in gently prepared DNA from human lymphoid cells in S phase (82) indicates that asymmetric DNA replication occurs in addition to simultaneous leading- and lagging-strand synthesis—which enzymes are involved in such replication? Furthermore, does the observed increase in DNA ligase II activity during meiotic prophase indicate a specific role for the enzyme in completing meiotic recombination reactions (27)? Is there a requirement in cells for DNA ligases that can act on the DNA·RNA hybrid structures occurring during transcription? Similarly, is a DNA ligase required to seal breaks in unusual DNA

Table 1 Properties of the three mammalian DNA ligases

	I	II	III
Molecular mass estimated by SDS-PAGE	125 kDa	72 kDa	100 kDa (associated with a 46-kDa polypeptide)
by cDNA sequence	102 kDa	—	—
Chromosomal localization	19q13.2–13.3	—	—
Ligation of oligo(dT) · poly(dA)	Yes	Yes	Yes
oligo(dT) · poly(rA)	No	Yes	Yes
oligo(rA) · poly(dT)	Yes	No	Yes
K_m for ATP	Low	High	Low
Adsorption to hydroxylapatite	Weak	Strong	Weak
Recognition by DNA ligase I–specific antisera	Yes	No	No
Subcellular localization	Nucleus	Nucleus	Nucleus/cytoplasm?
Induction upon cell proliferation	Yes	No	No
Proportion of DNA ligase activity in calf thymus extract	~85%	5–10%	5–10%

structures such as Z-DNA or H-DNA? Finally, joining of nonhomologous DNA molecules with protruding single-strand ends occurs efficiently in vertebrate cell extracts and is believed to be important during illicit recombination (83–85), but the DNA ligase(s) responsible for this reaction has not yet been identified. On a less speculative note, it should be mentioned that neither DNA ligase I, II, nor III is exclusively a mitochondrial enzyme (16, 27, 31; A. E. Tomkinson, T. Lindahl, unpublished data), but it is not known which of these three activities (if any) accounts for the circularization of mitochondrial DNA. A comparison of the properties of DNA ligases I, II, and III is shown in Table 1.

DNA LIGASES IN OTHER EUKARYOTIC SYSTEMS

Joining of Okazaki fragments during DNA replication is an essential cellular function, and ATP-dependent DNA ligases have been found to be ubiquitous

in eukaryotic cells. Early work documenting the presence of a DNA ligase in, for example, plant cells has been reviewed (39). Recent investigations have focused on well-characterized organisms that allow genetic or cell cycle analysis.

Schizosaccharomyces pombe *and* Saccharomyces cerevisiae *DNA Ligases*

The distantly related fission yeast and budding yeast each have an essential gene encoding a DNA ligase, *cdc17+* in *Sch. pombe* (86) and *CDC9* in *S. cerevisiae* (87). Conditional lethal DNA ligase mutants exhibit a phenotype typical of a cell division cycle (cdc) defect, and arrest in medial nuclear division at the restrictive temperature. Mutant cells contain an anomalously heat-labile DNA ligase activity and accumulate Okazaki fragments at 37 °C (87). *Sch. pombe cdc17* and *S. cerevisiae cdc9* mutants are also hypersensitive to ultraviolet light, ionizing radiation, and alkylating agents, indicating a general defect in DNA excision-repair in addition to the replication defect. The *S. cerevisiae CDC9* and *Sch. pombe cdc17+* genes have been cloned by functional complementation of a conditional lethal yeast DNA ligase mutant (88, 89), and a similar approach was later employed for the cloning of a complete human cDNA encoding DNA ligase I in *S. cerevisiae* (11). A comparison of the coding sequences (12) shows that the two yeast genes are related to each other and to human DNA ligase I (see Figure 1), indicating that they perform similar cellular functions. In particular, these three DNA ligases show strong sequence conservation towards their C-termini, whereas there is little or no sequence homology close to the N-termini. The highly conserved regions toward the C-termini of the DNA ligase polypeptides may define essential enzyme functions.

The *Sch. pombe cdc17+* gene encodes an 86-kDa protein (12, 90). Although the enzyme has not been highly purified, it can be detected as an 85–86-kDa enzyme-adenylate complex in crude cell extracts and partially purified protein fractions. Similar results have been obtained for the *S. cerevisiae CDC9* gene product. The 87-kDa primary translation product is readily degraded to an active 78-kDa fragment by intrinsic proteolysis in yeast cell extracts. Thus, the yeast DNA ligases seem to share a property of human DNA ligase I in having an N-terminal region that is not required for DNA ligase activity in vitro. However, the size of this apparently nonessential N-terminal region in yeast DNA ligases is only 1/3–1/2 of that of mammalian DNA ligase I.

In *S. cerevisiae*, *CDC9* and several other genes involved in DNA synthesis are periodically expressed at the G1/S boundary (91). However, such periodic transcription is not apparent for the *cdc17+* gene of *Sch. pombe* (92). The coordinate regulation of the relevant genes in *S. cerevisiae* is mediated by

binding of a specific transcription factor to an ACGCGT hexamer sequence occurring in the promoters of these genes (93). This factor may be a key regulatory element in the control of S phase entry in *S. cerevisiae.*

Transcription of the DNA ligase genes of *S. cerevisiae* and *Sch. pombe* is strongly induced by exposure to DNA-damaging agents (94, 95). However, dramatic changes in transcript levels are not matched by sizeable variations in the corresponding protein levels. DNA damage induces at most a 50% increase in DNA ligase activity in *S. cerevisiae*. This is probably due to the high level and long half-life of the enzyme in growing yeast cells. The increased transcription of the DNA ligase gene in response to DNA damage may be of greater importance in cells that have been in stationary phase for very long time periods (94).

The elegant and detailed molecular genetic studies of the major DNA ligase in yeast have not yet been extended by enzymological investigations and large-scale protein purification in an attempt to search for minor DNA ligase activities in these organisms. The question of whether yeast cells, like mammalian cells, contain more than one DNA ligase thus remains unresolved. Recent fractionation studies on yeast cell extracts have identified a second distinct DNA ligase activity that resembles the mammalian DNA ligases II and III in its ability to join an oligo (dT)·poly (rA) substrate (A. E. Tomkinson, unpublished data). In this regard, it is interesting that a DNA ligase–defective *cdc17* mutant of *Sch. pombe* with impaired DNA replication, repair, and mitotic recombination exhibits unaffected meiotic recombination (96). Higashitani et al (27) have proposed that mammalian DNA ligase II, rather than DNA ligase I, could serve to catalyze the final step in meiotic recombination. The yeast system offers the possibility of putting this speculative proposal of a defined function for DNA ligase II on a firm experimental footing.

Drosophila melanogaster *DNA Ligases*

Two distinct DNA ligases have been detected in *Drosophila.* DNA ligase I from *D. melanogaster* has been extensively characterized with regard to its mechanism of action (43, 97, 98). This enzyme, and DNA ligase I from bovine thymus, are the two most studied eukaryotic DNA ligases. The *Drosophila* DNA ligase I has been purified to apparent homogeneity from embryos. In common with DNA ligase I from other sources, it has a low K_M (1.6 μM) for ATP, is unable to join oligo (dT)·poly (rA), and performs blunt-end joining of DNA in the presence of polyethylene glycol. In addition, *Drosophila* DNA ligase I can ligate an oligo (rA)·poly (dT) hybrid substrate (43), as later also observed for mammalian DNA ligase I (45), but is unable to join oligo (rA)·poly (rU). Consequently, the substrate specificity of the *Drosophila* enzyme appears to be the same as for mammalian DNA ligase I, but

different from that of DNA ligases II and III. Detailed mechanistic studies have established that *Drosophila* DNA ligase I acts by precisely the same mechanism as that established for *E. coli* and bacteriophage T4 DNA ligases.

Drosophila DNA ligase I is isolated as an 83–86-kDa protein, as estimated by SDS-polyacrylamide gel electrophoresis, which is very susceptible to proteolysis and can be degraded by a variety of reagent proteases in vitro to an active fragment of 75 kDa, presumably by removal of N-terminal sequences. The 83–86-kDa form exhibits a high frictional ratio, indicating an asymmetric shape of the protein. Extensive proteolysis also generates a smaller 64-kDa form, which retains the ability to generate an enzyme-adenylate complex but can no longer catalyze the DNA-joining reaction. Such partially active proteolytic fragments have been observed upon digestion of *E. coli* DNA ligase (99) but have not been seen during limited proteolysis of mammalian DNA ligase I (15). These results indicate that *Drosophila* DNA ligase I is of a similar size to the corresponding *Sch. pombe* enzyme, although it cannot be excluded at present that the primary translation product is as large as the mammalian or *Xenopus* enzyme but is very rapidly cleaved to an 83–86-kDa active fragment in crude cell extracts. Cloning and sequencing of the *Drosophila* cDNA should resolve this problem. Alternatively, antibodies recognizing *Drosophila* DNA ligase I could be employed to define the size of the DNA ligase polypeptide by immunoblotting after gel electrophoresis of cell extracts prepared by instant lysis with hot SDS. Such detergent cell lysates were useful to define the correct size of the primary translation product of mammalian DNA ligase I (31).

A second DNA ligase with properties similar to those of mammalian DNA ligase II has been partially purified from *Drosophila* pupae and embryos (98, 100). In common with the mammalian enzyme, *Drosophila* DNA ligase II is not extracted from cell nuclei with an isotonic sucrose buffer of low ionic strength, and this accounts for an early unsuccessful attempt to detect the enzyme (97). However, *Drosophila* DNA ligase II is readily extracted from disrupted cells or isolated cell nuclei with 1 M NaCl, and the two *Drosophila* ligases can then be separated conveniently by phosphocellulose chromatography (98). The purified *Drosophila* DNA ligase II enzyme has a low affinity for the ATP cofactor and exhibits a K_M 10-fold higher than that of *Drosophila* DNA ligase I. Furthermore, the purified DNA ligase II can catalyze joining of an oligo (dT)·poly (rA) substrate. Two different inactivating monoclonal antibodies directed against *Drosophila* DNA ligase II have been isolated, and neither antibody binds or neutralizes *Drosophila* DNA ligase I (100). The *Drosophila* DNA ligase II is a 70-kDa protein, as defined both by immunoblotting and by analysis of a radioactive enzyme-AMP complex. In all these properties, the enzyme resembles mammalian DNA ligase II. Interestingly, a small amount of an apparent "larger form of DNA ligase II" has

also been observed during fractionation of *Drosophila* extracts (98, 100), hinting at the possibility that a third DNA ligase might be present in this organism.

Drosophila DNA ligase I is present at a high level in unfertilized eggs and early developing embryos, but at a 10-fold lower level in larvae, pupae, and adults (43, 98, 100). The DNA ligase I activity correlates well with changes in DNA replication during development, since a very high replication rate is characteristic of early embryos. In complete contrast, the level of DNA ligase II activity does not change significantly between different developmental stages. For this reason, the enzyme has been purified from pupae, where DNA ligase I levels are low. The availability of highly purified enzymes and appropriate antibodies provides a good starting point for attempts to define the functions of the different DNA ligases in *Drosophila*.

Xenopus laevis *DNA Ligase*

The dominant DNA ligase activity in oocytes and eggs of the clawed toad, *Xenopus laevis,* is due to a large enzyme of highly asymmetric shape, which is detected by antisera against mammalian DNA ligase I (101). The *Xenopus* DNA ligase cannot join an oligo (dT)·poly (rA) hybrid substrate, but catalyzes blunt-end joining of DNA. Similarly to DNA ligase I of *Drosophila*, the *Xenopus* enzyme is present at a 10-fold lower level in adult tissue than in eggs. The *Xenopus* DNA ligase has been purified to near homogeneity, and a specific antiserum against the enzyme obtained. However, the occurrence of further DNA ligases in this organism has not been investigated.

Molecular size determinations have been performed by SDS-polyacrylamide gel electrophoresis, either by analysis of a radioactive enzyme-AMP complex, or by immunoblotting with antibodies against the *Xenopus* enzyme or bovine DNA ligase I. The results indicate that the *Xenopus* DNA ligase is a 180-kDa protein, which can be rapidly degraded by proteolysis in vitro to active fragments of 130 kDa and then 76 kDa. These data show that the *Xenopus* enzyme has a 76-kDa catalytic domain, which is very similar in size to that present in mammalian DNA ligase I, *Drosophila* DNA ligase I, and the yeast DNA ligases. However, the native *Xenopus* enzyme is the largest DNA ligase observed to date. It seems likely that it has an even longer nonessential N-terminal region than mammalian DNA ligase I. Even if the *Xenopus* DNA ligase I shares the property of mammalian DNA ligase I of migrating anomalously slowly in SDS-polyacrylamide gels, it is still clearly larger than the bovine enzyme. DNA ligases have been partially purified from two other amphibians, *Pleurodeles* and axolotl (102). These are also large proteins of 180 kDa and 160 kDa, respectively, which are detected by an antibody directed against the *Xenopus* DNA ligase. In all three amphibians, the DNA ligase is found at a high level in oocytes, unfertilized eggs, and

embryos. A 25-kDa *Xenopus* DNA-binding protein that strongly promotes concatenation of linear DNA molecules by the DNA ligase has been identified (102a). Early reports of different size classes of DNA ligase before and after fertilization of amphibian eggs, as determined by sucrose gradient centrifugation of extracts (103, 104), probably reflected different levels of intrinsic proteolysis (101, 102), which converts the 180-kDa DNA ligase to the 76-kDa form. A second DNA ligase, if demonstrated in *Xenopus*, might be expected to occur at a similar level throughout development in analogy with *Drosophila* DNA ligase II. Injection of ultraviolet- or X-irradiated plasmid DNA into nuclei of *Xenopus* oocytes leads to rapid DNA repair with conversion of nicked circles to a covalently closed form (105, 106). It seems likely that the abundant DNA ligase accounts for this joining process.

Vaccinia Virus DNA Ligase

Human tumor viruses that encode a DNA polymerase, such as herpesviruses, adenoviruses, and retroviruses, do not produce a virus-specific DNA ligase. Thus, the complete DNA sequence of Epstein-Barr virus (107) does not contain an open reading frame with the active site motif (Figure 1) of a DNA ligase. Early investigations of vaccinia virus–infected cells demonstrated a 10-fold increase in DNA ligase activity in cell extracts (108), but failed to distinguish a virus-induced enzyme from host cell DNA ligase activity (109). However, recent studies (110–112) have clearly established that vaccinia virus encodes a DNA ligase. Since poxviruses, including vaccinia virus, have large genomes and replicate in the host cell cytoplasm, it is not surprising that they encode several different enzymes of DNA metabolism. The DNA ligase–encoding gene is transcribed early during infection. A restriction enzyme fragment carrying the relevant gene has been isolated and codes for a 63-kDa protein. The sequence of the vaccinia virus DNA ligase shows about 30% identity with those of the *Sch. pombe* and *S. cerevisiae* DNA ligases. The vaccinia enzyme is also distantly related to mammalian DNA ligase I, has a similar active site sequence (Figure 1), and contains a highly conserved peptide towards the C-terminus, which is also present in the C-terminal regions of mammalian DNA ligase I and the yeast DNA ligases (Figure 2). The vaccinia DNA ligase has been shown to form an enzyme-adenylate complex of about 61 kDa on incubation with ATP, in good agreement with the molecular mass estimate of the protein from the open reading frame. The adenylate residue is released from the enzyme-AMP complex on incubation with nicked DNA or with pyrophosphate, as expected for a DNA ligase. Moreover, the vaccinia enzyme catalyzes ligation of strand interruptions in an oligo (dT)·poly (dA) substrate.

The vaccinia virus–encoded DNA ligase is nonessential for viral DNA replication and growth on several types of host cells (112, 113). Possibly,

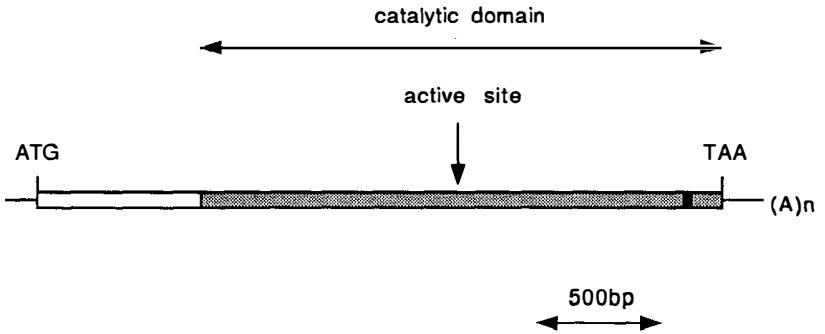

Figure 2 Schematic representation of the human DNA ligase 1 cDNA. Landmark features encoded by the cDNA are highlighted. The coding sequence is represented by an open box, the catalytic domain is shaded, and the position of a 16-amino-acid peptide, which is highly conserved in human DNA ligase I, as well as in the yeast and vaccinia DNA ligases (15), is marked by a solid bar.

vaccinia virus replicates its DNA in an asymmetric fashion without requirement for a DNA ligase (114), or traces of a host DNA ligase are recruited to the cytoplasmic viral replication factories. Recent studies have shown that a virus DNA ligase–deficient deletion mutant is anomalously sensitive to ultraviolet light and bleomycin during infection, indicating a role for the enzyme in viral DNA repair, and the mutant strain also exhibits attenuated virulence in vivo (77).

HUMAN CELL LINES DEFICIENT IN DNA LIGATION

Several human syndromes with autosomal recessive inheritance confer severe immunodeficiency and a greatly increased cancer frequency, while at the cellular level they are characterized by chromosome instability and hypersensitivity to DNA-damaging agents. These include (*a*) ataxia-telangiectasia (McKusick nr. 208900) and its two clinical variants (McKusick nrs. 208910 and 208920), and the closely related Nijmegen breakage syndrome (McKusick nr. 251260); (*b*) Fanconi's anemia (two genetic complementation groups, McKusick nrs. 227650 and 227660) and several variants (McKusick nrs. 227700, 227800, 227810, 227850), and the closely related Blackfan-Diamond anemia (McKusick nr. 205900); (*c*) Bloom's syndrome (McKusick nr. 210900). Direct measurements of enzyme activities in fractionated extracts of representative cell lines did not reveal any alteration of DNA ligases in ataxia-telangiectasia or Fanconi's anemia (115). However, Bloom's syndrome cells exhibit an apparent DNA ligation deficiency in vitro and in vivo (28, 78, 79, 115–117).

The DNA Ligase I–Defective Cell Line 46BR

A cell line with a DNA ligation deficiency was established from a young Irish female patient with clinical symptoms resembling Bloom's syndrome, that is, stunted growth, sun sensitivity, and severe immunodeficiency with recurrent infections. The patient died at age 19, apparently as a consequence of a slow-growing abdominal lymphoma in combination with an acute pulmonary infection. A fibroblast cell strain, 46BR, and an SV40-transformed line 46BR.1G1 with a closely similar phenotype, have been established in culture. 46BR cells are hypersensitive to a wide range of DNA-damaging agents including alkylating agents such as methyl methanesulfonate and dimethyl sulfate, ionizing radiation, and ultraviolet light. The cells are also hypersensitive to the poly ADP-ribose polymerase inhibitor 3-aminobenzamide, which interferes with DNA excision-repair. In contrast, ataxia-telangiectasia cells and Fanconi's anemia cells appear uniquely sensitive to ionizing radiation as opposed to DNA cross-linking agents and are normally resistant to methyl methanesulfonate and ultraviolet light. 46BR cells show a slightly but significantly elevated frequency of spontaneous sister chromatid exchange, and are hypersensitive to induction of such events by DNA-damaging agents. However, the spontaneous level of sister chromatid exchange is lower than that observed in Bloom's syndrome. This has been a main reason for considering 46BR as different from Bloom's syndrome, especially since measurements of sister chromatid exchange frequency are employed in the clinical diagnosis of Bloom's syndrome (59–63).

46BR fibroblasts, as well as the SV40-transformed subline, exhibit strongly delayed joining of Okazaki fragments during DNA replication (59, 60, 60a). Radioactive labeling of newly synthesized DNA in vivo with a 10-minute pulse of [^{3}H]thymidine, followed by DNA size determination by alkaline sucrose gradient centrifugation, showed that about one-half of the newly synthesized DNA remained as Okazaki fragments, whereas the great majority of newly replicated DNA in control cells was already in high-molecular-weight form. The delay in DNA joining observed in 46BR cells was only transient, because the newly synthesized DNA could be converted to a high-molecular-weight form during a 30-min chase period after the radioactive labeling. Furthermore, the rate of joining of DNA strand breaks occurring as a consequence of exposure of 46BR and 46BR.1G1 cells to dimethyl sulfate or ultraviolet light was markedly slower than that characteristic of control cells. These observations led to the proposal that 46BR cells might be defective in a DNA ligase (59, 63, 118).

Direct measurements of enzyme activities in cell extracts of 46BR.1G1, which grows better than 46BR in tissue culture, suggested similar levels of DNA-joining activity in standard in vitro DNA ligase assays for DNA ligases

I and II. However, DNA ligase I exhibited an unusual tendency to aggregate on size fractionation (60). Recent experiments have indicated that DNA ligase III activity is normal in 46BR.1G1 cells (58). Further investigations of the properties of DNA ligase I from 46BR.1G1 cells demonstrated a distinct anomaly, in that the amount of enzyme-adenylate complex formed on incubation with ATP is greatly reduced; only traces of the complex can be detected (58). In this regard, the DNA ligase I of 46BR.1G1 cells resembles the defective DNA ligase present in the conditional lethal *E. coli* mutant SG251 when grown at the permissive temperature; DNA-joining activity seems normal, but DNA ligase-AMP formation cannot be detected (32).

In a search for mutations in the structural gene encoding DNA ligase I in 46BR cells, the entire coding sequence was analyzed by direct DNA sequencing of cDNA amplified by the polymerase chain reaction (PCR) (58). In general, the coding sequence of the DNA ligase I gene is strongly conserved in the human population. Seven different DNA ligase I cDNA sequences from individuals of Anglo-Saxon, Ashkenazi Jewish, or Japanese origin did not reveal any amino acid replacements; the only polymorphisms observed were third-letter C/T variations in the codons for residues Gly377 and Asp802. However, diploid 46BR cells exhibited two sequence alterations in 50% of the cDNA. Both mutations represented CpG-to-TpG changes, a transition commonly found in human genetic disease (119) that may be ascribed to hydrolytic spontaneous deamination of a 5-methylcytosine residue. One of the mutations in the 46BR DNA ligase I cDNA would affect the active site region of the enzyme, leading to a replacement of the strongly conserved Glu566 residue (position −2 in Figure 1) with a Lys residue. Site-specific mutagenesis experiments (13) indicate that this alteration leads to an almost complete loss of enzyme activity. DNA ligase I protein synthesized in 46BR cells with the Glu566 → Lys alteration would be expected to be enzymatically inactive. The other mutation in 46BR DNA causes a replacement of Arg771, which is located in a region of the sequence showing strong evolutionary conservation, with a Trp residue. PCR amplification of appropriate sections of genomic DNA from 46BR cells and DNA sequencing established that both the mutations first seen in cDNA sequences are present in the cellular genome. Furthermore, genomic DNA from the mother of the patient contains the Arg771 → Trp mutation but not the Glu566 → Lys mutation. Thus, the two mutations seen in diploid 46BR cells are carried on different alleles, as also directly confirmed by cloning individual cDNAs, and one is maternally inherited. In the SV40-transformed aneuploid subline 46BR.1G1, selected as having the same hypersensitivity to DNA-damaging agents, gene rearrangements have occurred so that the line is homozygous for the Arg771 → Trp mutation but has the normal DNA sequence in the active site region. Consequently, the Arg771 → Trp mutation in DNA ligase I would appear to

account for the malfunctioning but partly active enzyme present in 46BR cells that allows cell proliferation.

Bloom's Syndrome

Several cases of Bloom's syndrome form a single genetic complementation group (120). Cells of Bloom's syndrome origin exhibit delayed joining of DNA replication intermediates (80, 121, 122). However, the replication intermediates that accumulate in vivo are not Okazaki fragments but larger intermediates of 20 kb. The rate of DNA replication is reduced, in particular in early S phase, and replication fork displacement rates are 55–65% of normal (123–125). After transfection of Bloom's syndrome cells with a linearized shuttle vector plasmid, recircularization by end-joining was reduced and error-prone compared to that observed in control cells, indicating a DNA ligation deficiency (117). Bloom's syndrome cells are moderately hypersensitive to many different types of DNA-damaging agents, but differ from 46BR cells in that a strong delay in joining of DNA strand breaks caused by dimethyl sulfate treatment does not occur (60).

DNA polymerases appear to be normal in Bloom's syndrome (126). In contrast, partially purified DNA ligase I from Bloom's syndrome cells exhibits reduced activity and altered fractionation properties, whereas DNA ligase II seems normal (28, 78, 79, 115, 116). In more detailed studies on the nucleic acid sequence level, PCR amplification and DNA sequencing of DNA ligase I cDNA from several different Bloom's syndrome cell lines has not revealed mutations within the coding sequence (58, 126a). The molecular explanation for the reduced DNA ligase activity in cell extracts of Bloom's syndrome origin remains to be firmly defined, but the following possibilities could be considered:

1. Measurements of DNA ligase activities in fractionated extracts of Bloom's syndrome cells were performed before DNA ligase III was identified (45). Thus, size fractionation of crude cell extracts to separate DNA ligases I and II yielded a high-molecular-weight active protein fraction of partially purified DNA ligase I, which in fact contained both DNA ligase I and DNA ligase III. Cloning and sequencing of cDNA sequences representative of DNA ligase III should now be performed, to investigate whether this activity is defective in Bloom's syndrome.

2. The activity of DNA ligase I is dependent on the phosphorylation state of the enzyme (18). The phosphorylation patterns and other posttranslational modifications of DNA ligase I from normal cells as compared to Bloom's syndrome cells need to be defined.

3. Several cellular protein factors that modulate the activity of DNA ligase I have been described. These include a ~65-kDa inhibitor that initially copurifies with DNA ligase I (67a) but is not present in the homogeneous calf

thymus enzyme (15). By comparison, purified DNA ligase III contains two different polypeptides, the larger of which carries the active site for adenylylation (16, 45). The DNA ligase I–associated protein factor has not yet been studied in Bloom's syndrome cells.

4. The alteration of DNA ligase I activity in Bloom's syndrome cell extracts could be the indirect consequence of a more general defect in cellular metabolism. For example, it has been proposed that Bloom's syndrome cells might be defective in detoxification of active oxygen species (127). The evidence for this is quite indirect, but includes an elevated cellular level of superoxide dismutase (128). The synthesis of a number of proteins seems to be abnormally regulated in Bloom's syndrome cells, including uracil-DNA glycosylase (129) and c-myc protein (130). However, the overproduction of c-myc protein may be related to the demonstrated induction of c-myc protein synthesis in response to the introduction of chain breaks in DNA (131).

The resolution of these alternatives has a bearing on the nomenclature and classification of the important DNA ligase I–defective 46BR cell line. If Bloom's syndrome is due to a mutation specifically affecting the molecular activity of a DNA ligase, 46BR could be considered a variant, or second genetic complementation group, of the syndrome. On the other hand, if the basic defect in Bloom's syndrome affects several cellular functions in addition to DNA ligation, 46BR might be regarded as the first representative of a novel human syndrome.

Acute Lymphoblastic Leukemia

Rusquet et al (132) reported that extracts of leukemic cells from patients with T-cell acute lymphoblastic leukemia had very low or undetectable levels of DNA ligase activity. These studies remain unconfirmed; a T-cell line representative of the disease, Jurkat/J-6, had normal levels of DNA ligase activities (133).

Concluding Statement

The biochemical defects in the inherited human syndromes associated with hypersensitivity to DNA-damaging agents remain largely unknown. Work is in progress in many laboratories to clone and characterize cDNA sequences that might complement the defects in diseases such as ataxia-telangiectasia, Fanconi's anemia, Cockayne's syndrome, and Werner's syndrome, and variants of these syndromes. With regard to the intensely studied and genetically complex disease, xeroderma pigmentosum, cDNA sequences for three of at least seven genes required for enzymatic incision at pyrimidine dimers in ultraviolet-irradiated DNA have been cloned. The sequences contain structural motifs indicating the presence of zinc fingers in the XP-A protein, and putative DNA helicase domains in the XP-B and XP-D proteins (37, 134–

136). However, the only protein purified to date is the XP-A protein, which binds specifically to ultraviolet-irradiated DNA (137). The isolated XP-A protein has no enzymatic activity by itself and presumably forms part of an incision complex. Thus, the single presently documented case within this group of inherited syndromes, in which a DNA sequence alteration can be correlated directly with a biochemical defect in an enzyme of known function, is represented by the individual from whom the 46BR cell line was established. The IgA and IgG deficiencies in this patient may be explained by inadequate DNA ligation during the recombinational processes in which V and C genes are joined, and antibody variability is induced by V gene processing. The hypersensitivity to DNA-damaging agents is undoubtedly due to inadequate DNA ligation during DNA excision repair. Interest in this general area has been furthered by the discovery that cells from severely immunodeficient mice homozygous for the *scid* mutation on chromosome 16 and defective in V(D)J [variable (diversity) joining] recombination are also hypersensitive to DNA damage introduced by ionizing radiation (138–141). Further investigation of cell lines derived from immunodeficient human individuals who are also hypersensitive to DNA-damaging agents promises new insights into the correlation between enzymes active in DNA metabolism and distinct forms of inherited disease.

Literature Cited

1. Li, J. J., Kelly, T. J. 1984. *Proc. Natl. Acad. Sci. USA* 81:6973–77
2. Wood, R. D., Robins, P., Lindahl, T. 1988. *Cell* 53:97–106
3. Jessberger, R., Berg, P. 1991. *Mol. Cell. Biol.* 11:445–57
4. Lehman, I. R. 1974. *Science* 186:790–97
5. Gumport, R. I., Lehman, I. R. 1971. *Proc. Natl. Acad. Sci. USA* 68:2559–63
6. Tomkinson, A. E., Totty, N. F., Ginsburg, M., Lindahl, T. 1991. *Proc. Natl. Acad. Sci. USA* 88:400–4
7. Montecucco, A., Lestingi, M., Pedrali-Noy, G., Spadari, S., Ciarrocchi, G. 1990. *Biochem. J.* 271:265–68
8. Uhlenbeck, O. C., Gumport, R. I. 1982. *Enzymes* 15B:31–58
9. Heaphy, S., Singh, M., Gait, M. J. 1987. *Biochemistry* 26:1688–96
10. Xu, Q., Teplow, D., Lee, T. D., Abelson, J. 1990. *Biochemistry* 29:6132–38
11. Barnes, D. E., Johnston, L. H., Kodama, K., Tomkinson, A. E., Lasko, D. D., Lindahl, T. 1990. *Proc. Natl. Acad. Sci. USA* 87:6679–83
12. Barker, D. G., White, J. H. M., Johnston, L. H. 1987. *Eur. J. Biochem.* 162:659–67
13. Kodama, K., Barnes, D. E., Lindahl, T. 1991. *Nucleic Acids Res.* 19: 6093–99
14. Teraoka, H., Tsukada, K. 1982. *J. Biol. Chem.* 257:4758–63
15. Tomkinson, A. E., Lasko, D. D., Daly, G., Lindahl, T. 1990. *J. Biol. Chem.* 265:12611–17
16. Elder, R. H., Rossignol, J. M. 1990. *Biochemistry* 29:6009–17
17. Mezzina, M., Rossignol, J. M., Philippe, M., Izzo, R., Bertazzoni, U., Sarasin, A. 1987. *Eur. J. Biochem.* 162:325–32
18. Prigent, C., Lasko, D. D., Kodama, K. I., Woodgett, J., Lindahl, T. 1992. Submitted
19. Siegel, L. M., Monty, K. J. 1966. *Biochim. Biophys. Acta* 112:346–62
20. Teraoka, H., Tsukada, K. 1985. *J. Biol. Chem.* 260:2937–40
21. Teraoka, H., Tsukada, K. 1986. *Biochim. Biophys. Acta* 873:297–303
22. Prigent, C., Aoufouchi, S., Philippe, M. 1990. *Biochem. Biophys. Res. Commun.* 169:888–95
23. Mezzina, M., Sarasin, A., Politi, N., Bertazzoni, U. 1984. *Nucleic Acids Res.* 12:5109–22

23a. Noguiez, P., Barnes, D. E., Mohren-

weiser, H. W., Lindahl, T. 1992. Submitted
24. Lindahl, T., Edelman, G. M. 1968. *Proc. Natl. Acad. Sci. USA* 61:680–87
25. Zimmerman, S. B., Levin, C. J. 1975. *J. Biol. Chem.* 250:149–55
26. Bhat, R., Grossman, L. 1986. *Arch. Biochem. Biophys.* 244:801–12
27. Higashitani, A., Tabata, S., Endo, H., Hotta, Y. 1990. *Cell Struct. Funct.* 15:67–72
28. Chan, J. Y. H., Becker, F. F. 1988. *J. Biol. Chem.* 263:18231–35
29. Yang, S. W., Becker, F. F., Chan, J. Y. H. 1990. *J. Biol. Chem.* 265:18130–34
30. Pedrali-Noy, G. C. F., Spadari, S., Ciarrocchi, G., Pedrini, A. M., Falaschi, A. 1973. *Eur. J. Biochem.* 39:343–51
31. Lasko, D. D., Tomkinson, A. E., Lindahl, T. 1990. *J. Biol. Chem.* 265:12618–22
32. Dermody, J. J., Robinson, G. T., Sternglanz, R. 1979. *J. Bacteriol.* 139:701–4
33. d'Arpa, P., Machlia, P. S., Ratrie, H., Rothfield, N. F., Cleveland, D. W., Earnshaw, W. C. 1988. *Proc. Natl. Acad. Sci. USA* 85:2543–47
34. Bestor, T., Laudano, A., Mattaliano, R., Ingram, V. 1988. *J. Mol. Biol.* 203:971–83
35. Barnes, D. E., Kodama, K., Tynan, K., Trask, B. J., de Jong, P. J., et al. 1992. *Genomics* 12:164–66
36. van Duin, M., deWit, J., Odijk, H., Westerveld, A., Yasui, A., et al. 1986. *Cell* 44:913–23
37. Weber, C. A., Salazar, E. P., Stewart, S. A., Thompson, L. H. 1990. *EMBO J.* 9:1437–47
38. Thompson, L. H., Brookman, K. W., Jones, N. J., Allen, S. A., Carrano, A. V. 1990. *Mol. Cell. Biol.* 10:6160–71
39. Soderhall, S., Lindahl, T. 1976. *FEBS Lett.* 67:1–8
40. Soderhall, S. 1975. *Eur. J. Biochem.* 51:129–36
41. Arrand, J. E., Willis, A. E., Goldsmith, I., Lindahl, T. 1986. *J. Biol. Chem.* 261:9079–82
42. Zimmerman, S. B., Pfeiffer, B. H. 1983. *Proc. Natl. Acad. Sci. USA* 80:5852–56
42a. Elder, R. H., Montecucco, A., Ciarrocchi, G., Rossignol, J. M. 1992. *Eur. J. Biochem.* 203:53–58
43. Rabin, B. A., Chase, J. W. 1987. *J. Biol. Chem.* 262:14105–11
44. Montecucco, A., Fontana, M., Focher, F., Lestingi, M., Spadari, S., Ciarrocchi, G. 1991. *Nucleic Acids Res.* 19:1067–72
45. Tomkinson, A. E., Roberts, E., Daly, G., Totty, N. F., Lindahl, T. 1991. *J. Biol. Chem.* 266:21728–35
46. Pinna, L. A. 1990. *Biochim. Biophys. Acta* 1054:267–84
47. Bensch, K. G., Tanaka, S., Hu, S.-Z., Wang, T. S.-F., Korn, D. 1982. *J. Biol. Chem.* 257:8391–96
48. Nakamura, H., Morita, T., Sato, C. 1986. *Exp. Cell Res.* 165:291–97
49. Nakayasu, H., Berezney, R. 1989. *J. Cell. Biol.* 108:1–11
50. Wilcock, D., Lane, D. P. 1991. *Nature* 349:429–31
51. Harlow, E., Whyte, P., Franza, B. R., Schley, C. 1986. *Mol. Cell Biol.* 6:1579–89
52. Whyte, P., Buchkovich, K. J., Horowitz, J. M., Friend, S. H., Raybuck, M., et al. 1988. *Nature* 334:124–29
53. Pines, J., Hunter, T. 1990. *Nature* 346:760–63
54. Goulian, M., Richards, S. H., Heard, C. J., Bigsby, B. M. 1990. *J. Biol. Chem.* 265:18461–71
55. Malkas, L. H., Hickey, R. J., Li, C., Pedersen, N., Baril, E. F. 1990. *Biochemistry* 29:6362–74
56. Soderhall, S., Lindahl, T. 1975. *J. Biol. Chem.* 250:8438–44
57. Soderhall, S. 1976. *Nature* 260:640–42
58. Barnes, D. E., Tomkinson, A. E., Lehmann, A. R., Webster, D., Lindahl, T. 1992. *Cell.* In press
59. Henderson, L. M., Arlett, C. F., Harcourt, S. A., Lehmann, A. R., Broughton, B. C. 1985. *Proc. Natl. Acad. Sci. USA* 82:2044–48
60. Lehmann, A. R., Willis, A. E., Broughton, B. C., James, M. R., Steingrimsdottir, H., et al. 1988. *Cancer Res.* 48:6343–47
60a. Lönn, U., Lönn, S., Nylen, U., Winblad, G. 1989. *Carcinogenesis* 10:981–85
61. Webster, D., Arlett, C. F., Harcourt, S. A., Teo, I. A., Henderson, L. 1982. In *Ataxia-telangiectasia—A Cellular and Molecular Link between Cancer, Neuropathology, and Immune Deficiency*, ed. B. A. Bridges, D. G. Harnden, pp. 379–86. New York: Wiley
62. Teo, I. A., Arlett, C. F., Harcourt, S. A., Priestley, A., Broughton, B. C. 1983. *Mutat. Res.* 107:371–86
63. Teo, I. A., Broughton, B. C., Day, R. S., James, M. R., Karran, P., Mayne, L. V., Lehmann, A. R. 1983. *Carcinogenesis* 4:559–64
64. Sancar, A., Sancar, G. B. 1988. *Annu. Rev. Biochem.* 57:29–67
65. Spadari, S., Ciarrocchi, G., Falaschi, A. 1971. *Eur. J. Biochem.* 22:75–78
66. Kenne, K., Ljungquist, S. 1988. *Eur. J. Biochem.* 174:465–70

67. Scher, B. M., Scher, W., Waxman, S. 1988. *Cancer Res.* 48:6278–84
67a. Yang, S. W., Becker, F. F., Chan, J. Y. H. 1992. *Proc. Natl. Acad. Sci. USA*. In press
68. Creissen, D., Shall, S. 1982. *Nature* 296:271–72
69. Ueda, K., Hayaishi, O. 1985. *Annu. Rev. Biochem.* 54:73–100
69a. Yang, S. W., Huang, P., Plunkett, W., Baker, F. F., Chan, J. Y. H. 1992. *J. Biol. Chem.* In press
70. Chan, J. Y. H., Becker, F. F. 1985. *Carcinogenesis* 6:1275–77
71. Teraoka, H., Sumikawa, T., Tsukada, K. 1986. *J. Biol. Chem.* 261:6888–92
72. Lasko, D. D., Tomkinson, A. E., Lindahl, T. 1990. *Mutat. Res.* 236:277–87
73. Gould, K. L., Bretscher, A., Esch, F. S., Hunter, T. 1989. *EMBO J.* 8:4133–42
74. Turunen, O., Winqvist, R., Pakkanen, R., Grzeschik, K.-H., Wahlstrom, T., Vaheri, A. 1989. *J. Biol. Chem.* 264: 16727–32
75. Bretscher, A. 1986. *Methods Enzymol.* 134:24–37
76. Bretscher, A. 1989. *J. Cell. Biol.* 108: 921–30
77. Kerr, S. M., Johnston, L. H., Odell, M., Duncan, S. A., Law, K. M., Smith, G. L. 1991. *EMBO J.* 10:4343–50
78. Chan, J. Y. H., Becker, F. F., German, J., Ray, J. H. 1987. *Nature* 325:357–59
79. Willis, A. E., Weksberg, R., Tomlinson, S., Lindahl, T. 1987. *Proc. Natl. Acad. Sci. USA* 84:8016–20
80. Lönn, U., Lönn, S., Nylen, U., Winblad, G., German, J. 1990. *Cancer Res.* 50:3141–45
81. Blackburn, E. H. 1991. *Nature* 350: 569–73
82. Bjursell, G., Gussander, E., Lindahl, T. 1979. *Nature* 280:420–23
83. Roth, D. B., Wilson, J. H. 1986. *Mol. Cell. Biol.* 6:4295–304
84. Thode, S., Schäfer, A., Pfeiffer, P., Vielmetter, W. 1990. *Cell* 60:921–28
85. North, P., Ganesh, A., Thacker, J. 1990. *Nucleic Acids Res.* 18:6205–10
86. Nasmyth, K. A. 1977. *Cell* 12:1109–20
87. Johnston, L. H., Nasmyth, K. A. 1978. *Nature* 274:891–93
88. Barker, D. G., Johnston, L. H. 1983. *Eur. J. Biochem.* 134:315–19
89. Johnston, L. H., Barker, D. G., Nurse, P. 1986. *Gene* 41:321–25
90. Banks, G. R., Barker, D. G. 1985. *Biochim. Biophys. Acta* 826:180–85
91. Johnston, L. H. 1990. *Curr. Opin. Cell Biol.* 2:274–79
92. White, J. H. M., Barker, D. G., Nurse, P., Johnston, L. H. 1986. *EMBO J.* 5:1705–9
93. Lowndes, N. F., Johnson, A. L., Johnston, L. H. 1991. *Nature* 350:247–50
94. Johnson, A. L., Barker, D. G., Johnston, L. H. 1986. *Curr. Genet.* 11:107–12
95. Peterson, T. A., Prakash, L., Prakash, S., Osley, M. A., Reed, S. I. 1985. *Mol. Cell. Biol.* 5:226–35
96. Sipiczki, M., Grossenbacher-Grunder, A.-M., Bódi, Z. 1990. *Mol. Gen. Genet.* 220:307–13
97. Rabin, B. A., Hawley, R. S., Chase, J. W. 1986. *J. Biol. Chem.* 261:10637–45
98. Takahashi, M., Senshu, M. 1987. *FEBS Lett.* 213:345–52
99. Panasenko, S. M., Modrich, P., Lehman, I. R. 1976. *J. Biol. Chem.* 251:3432–35
100. Takahashi, M., Tomizawa, K. 1990. *Eur. J. Biochem.* 192:735–40
101. Hardy, S., Aoufouchi, S., Thiebaud, P., Prigent, C. 1991. *Nucleic Acids Res.* 19:701–5
102. Aoufouchi, S., Hardy, S., Prigent, C., Philippe, M., Thiebaud, P. 1991. *Nucleic Acids Res.* 19:4395–98
102a. Bayne, M. L, Alexander, R. F., Benbow, R. M. 1984. *J. Mol. Biol.* 172:87–108
103. Signoret, J., David, J. C. 1986. *Int. Rev. Cytol.* 103:249–79
104. Signoret, J., David, J. C., Lefresne, J., Jouillon, C. 1983. *Proc. Natl. Acad. Sci. USA* 80:3368–71
105. Legerski, R. J., Penkala, J. E., Peterson, C. A., Wright, D. A. 1987. *Mol. Cell. Biol.* 7:4317–23
106. Sweigert, S. E., Carroll, D. 1990. *Mol. Cell. Biol.* 10:5849–56
107. Baer, R., Bankier, A. T., Biggin, M. D., Deininger, P. L., Farrell, P. J., et al. 1984. *Nature* 310:201–11
108. Sambrook, J., Shatkin, A. J. 1969. *J. Virol.* 4:719–26
109. Spadari, S. 1976. *Nucleic Acids Res.* 3:2155–67
110. Kerr, S. M., Smith, G. L. 1989. *Nucleic Acids Res.* 17:9039–50
111. Smith, G. L., Chan, Y. S., Kerr, S. M. 1989. *Nucleic Acids Res.* 17:9051–62
112. Colinas, R. J., Goebel, S. J., Davis, S. W., Johnson, G. P., Norton, E. K., Paoletti, E. 1990. *Virology* 179:267–75
113. Kerr, S. M., Smith, G. L. 1991. *Virology* 180:625–32
114. Moss, B. 1990. *Annu. Rev. Biochem.* 59:661–88
115. Willis, A. E., Lindahl, T. 1987. *Nature* 325:355–57
116. Kurihara, T., Teraoka, H., Inoue, M.,

Takebe, H., Tatsumi, K. 1991. *Jpn. J. Cancer Res.* 82:51–57
117. Rünger, T. M., Kraemer, K. H. 1989. *EMBO J.* 8:1419–425
118. Squires, S., Johnson, R. T. 1983. *Carcinogenesis* 4:565–72
119. Cooper, D. N., Youssoufian, H. 1988. *Hum. Genet.* 78:151–55
120. Weksberg, R., Smith, C., Anson-Cartwright, L., Maloney, K. 1988. *Am. J. Hum. Genet.* 42:816–24
121. Giannelli, F., Benson, P. F., Pawsey, S. A., Polani, P. E. 1977. *Nature* 265:466–69
122. Ockey, C. H., Saffhill, R. 1986. *Carcinogenesis* 7:53–57
123. Hand, R., German, J. 1975. *Proc. Natl. Acad. Sci. USA* 72:758–62
124. Kapp, L. N. 1982. *Biochim. Biophys. Acta* 696:226–27
125. Fujikawa-Yamamoto, K., Odashima, S., Kurihara, T., Murakami, F. 1987. *Cell Tissue Kinet.* 20:69–76
126. Spanos, A., Holliday, R., German, J. 1986. *Hum. Genet.* 73:119–22
126a. Petrini, J. H. J., Huwiler, K. G., Weaver, D. T. 1991. *Proc. Natl. Acad. Sci. USA* 88:7615–19
127. Hirschi, M., Netrawali, M. S., Remsen, J. F., Cerutti, P. A. 1981. *Cancer Res.* 41:2003–7
128. Nicotera, T. M., Notaro, J., Notaro, S., Schumer, J., Sandberg, A. A. 1989. *Cancer Res.* 49:5239–43
129. Vollberg, T. M., Seal, G., Sirover, M. A. 1987. *Carcinogenesis* 8:1725–29
130. Sullivan, N. F., Willis, A. E., Moore, J. P., Lindahl, T. 1989. *Oncogene* 4:1509–11
131. Sullivan, N. F., Willis, A. E. 1989. *Oncogene* 4:1497–502
132. Rusquet, R. M., Feon, S. A., David, J. C. 1988. *Cancer Res.* 48:4038–44
133. Lindahl, T., Willis, A. E., Lasko, D. D., Tomkinson, A. 1989. In *DNA Repair Mechanisms and their Biological Implications in Mammalian Cells,* ed. M. Lambert, J. Laval, pp. 429–38. New York:Plenum
134. Tanaka, K., Miura, N., Satokata, I., Miyamoto, I., Yoshida, M. C., et al. 1990. *Nature* 348:73–76
135. Weeda, G., van Ham, R. C. A., Vermeulen, W., Bootsma, D., van der Eb, A. J., Hocijmakers, J. H. J. 1990. *Cell* 62:777–91
136. Fleiter, W. L., McDaniel, L. D., Johns, D., Friedberg, E. C., Schulz, R. A. 1992. *Proc. Natl. Acad. Sci. USA* 89:261–65
137. Robins, P., Jones, C. J., Biggerstaff, M., Lindahl, T., Wood, R. D. 1991. *EMBO J.* 10:3913–21
138. Fulop, G. M., Phillips, R. A. 1990. *Nature* 347:479–82
139. Biedermann, K. A., Sun, J., Giaccia, A. J., Tosto, L. M., Brown, J. M. 1991. *Proc. Natl. Acad. Sci. USA* 88:1394–97
140. Hendrickson, E. A., Qin, X.-Q., Bump, E. A., Schatz, D. G., Oettinger, M., Weaver, D. T. 1991. *Proc. Natl. Acad. Sci. USA* 88:4061–65
141. Hendrickson, E. A., Lin, V. F., Weaver, D. T. 1991. *Mol. Cell. Biol.* 11:3155–62
142. Lauer, G., Rudd, E. A., McKay, D. L., Ally, A., Ally, D., Backman, K. C. 1991. *J. Bacteriol.* 173:5047–53

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