Cloned Mouse Ribonucleotide Reductase Subunit M1 cDNA Reveals Amino Acid Sequence Homology with Escherichia coli and Herpesvirus Ribonucleotide Reductases

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We have isolated and sequenced overlapping cDNA clones containing the entire coding region of mouse ribonucleotide reductase subunit M1. The coding region comprises 2.4 kilobases and predicts a polypeptide of 792 amino acids (M, 90,234) which shows striking homology with ribonucleotide reductases from Escherichia coli and the herpesviruses, Epstein-Barr virus and herpes simplex virus. The homology reveals three domains: an N-terminal domain common to the mammalian and bacterial enzymes, a C-terminal domain common to the mammalian and viral ribonucleotide reductases, and a central domain common to all three. We speculate on the functional basis of this conservation.

Ribonucleotide reductase is a crucial cell enzyme, providing the only route for de novo synthesis of deoxynucleotide substrates for DNA replication via direct reduction of the corresponding ribonucleoside diphosphates. Mammalian ribonucleotide reductase is composed of two nonidentical subunits, M1 and M2, with molecular weights of 90,000 and approximately 55,000, respectively (Thelander and Reichard, 1979). The enzyme is under complex allosteric control mediated by the binding of deoxynucleoside triphosphates and ATP to binding sites on the M1 subunit. Two distinct regulatory sites have been defined: the specificity site, which controls substrate specificity, and the activity site, which regulates overall catalytic activity (Thelander and Reichard, 1979; Eriksson et al., 1981a). Photoaffinity labeling experiments have localized a third site on M1, a substrate-binding catalytic site, formed only in the presence of the second subunit, M2 (Caras et al., 1983). We have previously characterized mutants of ribonucleotide reductase subunit M1 that carry alterations in the allosteric sites that specifically disrupt the binding of one or more nucleotide effectors (Eriksson et al., 1981a). One of these mutants (an activity site mutant) is resistant to feedback inhibition by dATP, producing elevated deoxynucleotide pools and a mutator phenotype in cells (Weinberg et al., 1981).

In addition to complex structure-function relationships, ribonucleotide reductase presents a number of interesting problems in terms of growth regulation and human disease.

The level of enzyme activity is closely correlated with the growth rate of a cell (Elford et al., 1970) and appears to vary within the cell cycle (Erikssom and Martin, 1981; Eriksson et al., 1984). Ribonucleotide reductase is also thought to mediate the pathogenesis of the immunodeficiency that results from an inherited deficiency of adenosine deaminase or purine nucleoside phosphorylase (Martin and Gelfand, 1981). The deoxynucleotides that accumulate in the lymphoid cells of these patients are thought to feedback inhibit ribonucleotide reductase, preventing DNA replication and cell proliferation.

In this paper, we report the isolation of three overlapping cDNA clones, spanning 2.9 kilobases, which include the entire coding region of mouse ribonucleotide reductase subunit M1. The mRNA used to generate these clones was from a mutant T-lymphoma (S49) cell line which expresses a dATP feedback-resistant mutant form of M1. We have compared the deduced primary amino acid sequence of the M9,90,000 subunit M1 with the recently published sequences of the analogous Escherichia coli subunit B1 (Carlson et al., 1984) and the ribonucleotide reductases of the herpesviruses, Epstein-Barr virus (EBV) (Gibson et al., 1984) and herpes simplex virus (HSV) (McLachlan and Clements, 1983; Dutia, 1983; Baccetti et al., 1984). We report here a striking similarity in specific regions of the sequence and propose that this homology may be functionally relevant.

**EXPERIMENTAL PROCEDURES**

Cell Lines and Antiserum—Mouse T-lymphoma cells, S49 (Hori-bata and Harris, 1970) or WEHI-7 (Harris et al., 1973), were grown in Dulbecco's modified Eagle's medium with 10% heat-inactivated horse serum. The isolation and characterization of the S49 cell mutant, dGuo-200-1-A, will be described elsewhere. A polyclonal anti-M1 rabbit serum was raised against S49 cell subunit M1 purified to homogeneity by affinity chromatography on dextran blue-Sepharose and dATP-Sepharose (Eriksson et al., 1981b). IgG was isolated on DEAE-Affi-Gel Blue (Bio-Rad) using conditions recommended by the supplier.

Polysome Purification and RNA Isolation—Polysomes were prepared from exponentially growing S49 or WEHI-7 cells as described by Goddard et al. (1983). Immunoabsorption of polysomes with anti-M1 antibody, isolation of the antibody-antigen complex by protein A-Sepharose chromatography, and extraction of the poly(A)* RNA were as described by Kraus and Rosenburg (1982). Total cytoplasmic poly(A)* RNA from S49 or WEHI-7 cells was prepared as described (Goddard et al., 1983).

**In Vitro Translation Assays—**In vitro translation was carried out in the presence of [35S]methionine (Amersham Corp.) using a rabbit

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1 The abbreviations used are: EBV, Epstein-Barr virus; HSV, herpes simplex virus; kb, kilobases.

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reticulocyte lysate (Amersham Corp. or Promega Biotec) according to conditions recommended by the supplier. Reaction products were immunoprecipitated according to the procedure of Firestone et al. (1982), except that only one round of immunoprecipitation was performed and formalin-fixed *Staphylococcus aureus* A cells (Pansorbin) were purchased from Calbiochem-Behring. Chromatography of the reaction products on dextran blue-Sepharose and/or dATP-Sepharose was carried out as previously described (Eriksson et al., 1981b) using 0.1-ml columns. Fractions were concentrated by precipitation with trichloroacetic acid in the presence of bovine serum albumin and analyzed by sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis (Laemmli, 1970).

Construction and Screening of cDNA Libraries—Two cDNA libraries were constructed. Initially, double-stranded cDNA was prepared from 100 ng of M1-enriched S49 cell mRNA primed with oligo(dT) as described by Goddard et al. (1983). The double-stranded cDNA was inserted into the *PsI* site of pBR322 by dC/dG homopolymeric tailing and used to transform *E. coli* strain K12 MC1061 (Goddard et al., 1983). The resultant clones were screened by colony hybridization (Gerken et al., 1979) using 32P-labeled single-stranded cDNA probes reverse-transcribed from enriched or unfractonated WEHI-7 poly(A)+ RNA.

For the second library, 0.8 μg of a specific 18-mer DNA primer was mixed with 10 μg of unfractonated polysomal Poly(A)+ RNA, heated to 70 °C for 3 min, and then chilled on dry ice. The reaction mixture was adjusted to 20 mM Tris-HCl (pH 8.5), 20 mM KCl, 8 mM MgCl₂, 25 mM dithiothreitol, 0.5 mM of each deoxynucleoside triphosphate, 1 μCi/μl [α-32P]dCTP, 4 mM sodium pyrophosphate, and 2 units/μl RNasin (Promega Biotec) in a final volume of 100 μl. cDNA synthesis was initiated by the addition of 50 units of reverse transcriptase and incubation at 42 °C for 20 min. The cDNA was made double-stranded by standard procedures (Pennaeta et al., 1983; Goddel, 1980) and electroforeased on a 6% polyacrylamide gel. Two-hundred nanograms of double-stranded cDNA (greater than 600 base pairs in length) was recovered by electroelution, ligated to a synthetic adapter, and used to transform *E. coli* strain K12 MC1061 (Goddard et al., 1983). The DNA was packaged in vitro using the Packagene extract from Promega Biotec and plated onto Y1073, an Hfl derivative of C600 (Young and Davis, 1983). The recombinant plaques were screened as described by Maniatis et al. (1982) using a 32P-labeled nick-translated cDNA fragment of p201 (see “Results”).

Identification of Clones by Hybrid Selection—Positive hybrid selection was performed essentially as described by Maniatis et al. (1983). Recombinant plasmid DNA (6 μg) bound to diazobenzyloxymethyl paper (S and S Transa-Bind, aminophenylthioether form) was hybridized with 75 μg of Poly(λ')RNA. The hybridized mRNA was

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**FIG. 1. Identification of M1-specific mRNA by in vitro translation followed by affinity chromatography or immunoprecipitation.** Poly(A)+ RNA from S49 cells was translated in a reticulocyte cell-free system containing [35S]methionine and treated as follows. a, 5% of the labeled translation products was loaded directly onto a sodium dodecyl sulfate-10% polyacrylamide gel (lane 1). The remainder was fractionated on dextran blue-Sepharose, and the bound proteins eluted with 0.25 M KCl, 0.05 M Tris-HCl, pH 7.5, 1 mM dithiothreitol. Aliquots of the flow-through (lane 2) and bound (lane 3) fractions were analyzed by gel electrophoresis. The proteins that bound to dextran blue-Sepharose were further fractioned on dATP-Sepharose. Bound proteins were eluted with 30 mM ATP in 0.05 M Tris-HCl, pH 7.5, 0.01 M KCl, 1 mM dithiothreitol and analyzed by gel electrophoresis. Lane 4, flow-through fraction; lane 5, bound fraction. The arrowhead indicates the position of M1 determined by co-electrophoresis with purified subunit M1 from S49 cells (not shown). b, immunoprecipitation of 35S-labeled, translated proteins by incubation with preimmune serum (lane 6) or anti-M1 antibody (lane 7) followed by adsorption to formalin-fixed *S. aureus* A cells (Firestone et al., 1982) and analysis by gel electrophoresis.

**FIG. 2. Analysis of M1-enriched mRNA from immunoadsorbed polysomes.** The mRNA extracted from either immunoadsorbed (lanes I) or unfraccionated (lanes U) polysomes was translated in vitro. Two percent of the labeled translation products was loaded directly onto a sodium dodecyl sulfate-polyacrylamide gel (a). The remainder was subjected either to immunoprecipitation with anti-M1 antibody (b) or to affinity chromatography on dATP-Sepharose (c). Polysomes were prepared from WEHI-7 cells (a and b) or S49 cells (c).
eluted in two changes of 200 μl of 1 mM EDTA, 5 μg of tRNA at 80 °C for 1 min. The eluted mRNA was ethanol-purified and assayed by in vitro translation and immunoprecipitation.

Sequence Analysis—DNA nucleotide sequences were determined by the dideoxyribonucleotide chain termination method (Smith, 1980) after subcloning of appropriate restriction fragments into derivatives of bacteriophage M13 (Messing et al., 1981). A partial N-terminal sequence of purified subunit M1 (Eriksson et al., 1981b) was determined by the Edman method (Edman and Begg, 1967) using a modified Beckman microsequenator.

Other Methods—Plasmid and bacteriophage DNAs were prepared essentially as described by Maniatis et al. (1982).

Double-stranded DNA was labeled by nick translation (Rigby et al., 1977) to a specific activity of 10^7–10^8 cpm/μg.

For Southern analysis (Southern, 1975), prehybridization was performed at 42 °C in 50% formamide, 0.05 M NaPO₄, pH 6.8, 5 × SSC, 5 × Denhardt’s solution (Maniatis et al., 1982), and 150 μg/ml salmon sperm DNA. Hybridization solutions contained, in addition, 10% dextran sulfate and 32P-labeled probe (2.5 × 10⁶ cpm/ml). Filters were washed at 60 °C in 0.2 × SSC, 0.1% sodium dodecyl sulfate before autoradiography.

The oligonucleotide primer was synthesized by the solid-phase phosphotriester method (Crea and Horn, 1980).

RESULTS

The poly(A)^+ RNA used to generate the cDNA clones described below was from a mutant S49 cell line, dGuo-200-1.2 These cells have the following properties: 1) they are resistant to deoxyadenosine toxicity; 2) they contain altered ribonucleotide reductase subunit M1 molecules which show reduced affinity for dATP at the activity site and do not respond to normal feedback regulation by dATP; and 3) they appear to express predominantly one protein M1 allele, the mutant allele.

Cloning Strategy—Protein M1 represents less than 0.05% of the total cell protein in S49 cells.3 Assuming the mRNA to be of similarly low abundance, we enriched the mRNA approximately 100-fold by immunoadsorbing polysomes with anti-M1 antibody. The enriched mRNA was used to construct a cDNA library, and potential M1 clones were selected by a differential screening procedure (see below). M1 clones were then identified by positive hybrid selection. Since the initial clones lacked the full coding region, a second cDNA library was constructed by specific priming followed by cloning into a Agt10 vector.

Detection and Enrichment of M1 mRNA—To follow the enrichment of M1 mRNA and to facilitate identification of M1 clones by hybrid selection, we developed two independent assays for M1-specific RNA sequences. After in vitro translation of S49 cell poly(A)^+ RNA, 35S-labeled proteins (Fig. 1, lane 1) were immunoprecipitated with anti-M1 antibody (lane 2).

a. Immunoprecipitated from S49 poly(A)^+ RNA.

b. Immunoprecipitated from WEHI-7 poly(A)^+ RNA.

c. Immunoprecipitated from WEHI-7 poly(A)^+ RNA subjected to hybrid selection.

7) or subjected to affinity chromatography on dextran blue-Sepharose followed by dATP-Sepharose (lane 5) and analyzed on sodium dodecyl sulfate-polyacrylamide gels. Both procedures lead to the substantial enrichment of a 90-kDa protein which co-migrates with authentic M1 (data not shown) and has identical chromatographic properties. A control with preimmune serum indicated that M1 immunoprecipitation was specific (compare lanes 6 and 7). Because S49 cells contained a mouse mammary tumor virus protein that was...
weakly precipitated by the anti-M1 antibody (protein V, lane 7), we used mRNA from WEHI-7 cells (which do not express mouse mammary tumor virus (Stallcup et al., 1978)) to generate cDNA probes used in the screening of cDNA libraries as described below.

To prepare M1-enriched mRNA, we used polysomes (S49 or WEHI-7) purified by immunoadsorption with anti-M1 antibody followed by chromatography on protein A-Sepharose. In vitro translation of the enriched mRNA produced one major 90-kDa protein (Fig. 2, lane 1) which was immunoprecipitable with anti-M1 antibody as expected (lane 3) and also bound to dATP-Sepharose (lane 5), providing independent evidence that the immunoadsorbed polysomal mRNA was enriched for M1-specific mRNA. Densitometric scanning of the bands in lane 1 suggested that M1-specific mRNA comprised approximately 5% of the total polysome-enriched mRNA preparation, representing a 100-fold enrichment.

**Molecular Cloning of Ribonucleotide Reductase Subunit M1 cDNA—**The initial library, prepared from M1-enriched mRNA cloned into the PstI site of pBR322, contained approximately 2000 tetracycline-resistant, ampicillin-sensitive transformants. Potential ribonucleotide reductase subunit M1 clones were identified by differential colony screening using [32P]cDNA probes transcribed from poly(A)+ RNA from lymphoma cells, representing a 100-fold enrichment. Approximately 50 colonies (2.5%) exhibited the required pattern suggesting that M1-specific mRNA comprised only to cDNA made from the M1-enriched mRNA. Approximately 20 colonies (2.5%) exhibited the required pattern, indicative of the 3' polyadenylation signal, AATAAA, is underlined. Approximately 20 colonies (2.5%) exhibited the required pattern, indicative of the 3' polyadenylation signal, AATAAA, is underlined.
tion with BarnHI, fractionated, and transferred to nitrocellulose by the Southern procedure (Southern, 1975). DNA bands homologous to cDNA inserts were examined by hybrid selection as described below. We identified two recombinant M1 plasmids, designated p201 and p247, which contained cDNA inserts of 1.6 and 1.2 kb, respectively. Restriction analysis showed that they contain a 700-base pair overlap region and together span 2.1 kb (Fig. 3). To obtain clones extending in the 5′ direction, we used a specific 18-mer (dTGGTTAGTCTCCACTCGC, complementary to a region of M1 mRNA 300 base pairs from the poly(A) tail of the mRNA, preceded by the eukaryotic polyadenylation signal, AATAAA (Proudfoot and Brownlee, 1976), was present at one end of p247. This allowed us to orient the sequences of the cDNA clones with that of the mRNA. These contiguous clones contained a single open reading frame encoding a 90-kDa protein (Fig. 5). To provide definitive proof that this sequence encodes M1, 15 of the first 18 amino acids of purified subunit M1 were determined by reference to a HindIII digest of bacteriophage λ DNA.

Identification of Ribonucleotide Reductase Subunit M1 cDNA Clones by Hybrid Selection—In order to determine if the cDNA clones isolated by differential screening contained M1 cDNA sequences, hybrid selection analysis was performed. Potential recombinant M1 plasmids were bound to diazobenzyloxymethyl paper filters and incubated with poly(A)+ RNA from either S49 or WEHI-7 cells. The hybridized mRNAs were eluted and translated in vitro. Two per cent of the translation products was analyzed directly by sodium dodecyl sulfate gel electrophoresis (Fig. 4a), while the remainder was subjected to immunoprecipitation prior to electrophoresis (Fig. 4, b and c). Lanes 11, 19, and 21 of Fig. 4 show that p201 and p247 specifically selected an mRNA encoding a 90-kDa protein immunoprecipitable with anti-M1 antibody. This mRNA was selected from both S49 and WEHI-7 poly(A)+ RNA.

DNA Sequence Analysis and Proof of the Identity of M1 cDNA Clones—Fig. 3 shows the strategy used for sequence analysis of the cDNA inserts of p247, p201, and M1-1. A stretch of at least 30 adenosine residues, presumably corresponding to the poly(A) tail of the mRNA, preceded by the eukaryotic polyadenylation signal, AATAAA (Proudfoot and Brownlee, 1976), was present at one end of p247. This allowed us to orient the sequences of the cDNA clones with that of the mRNA. These contiguous clones contained a single open reading frame encoding a 90-kDa protein (Fig. 5). To provide definitive proof that this sequence encodes M1, 15 of the first 18 amino acids of purified subunit M1 were determined. As shown in Fig. 5, these matched exactly with the N terminus predicted by the cDNA.

The mRNA used to generate the cDNA clones described above was isolated from mutant S49 cells that appear to express predominantly a dATP feedback-resistant form of M1. To determine whether the sequence shown in Fig. 5 represents dATP-resistant or wild-type M1, we isolated and sequenced an M1 cDNA from mutant S49 cells that express both wild-type and dGTP/dTTP-resistant M1 (Ullman et al., 1981) (data not shown). This sequence was identical to that shown in Fig. 5, suggesting that it represents wild-type M1.

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Amino Acid Sequence Homology between Ribonucleotide Reductase from Mouse, E. coli, and the Herpesviruses, EBV and HSV-2—We compared the deduced amino acid sequence of mouse M1 with that of the analogous E. coli subunit B1 (Carlson et al., 1984) and with the putative 93-kDa ribonucleotide reductase of EBV (Gibson et al., 1984). (Since the complete sequence of the HSV 140-kDa ribonucleotide reductase gene has not been determined, we used the homologous EBV 93-kDa gene for the alignment.) Fig. 7 shows an alignment of the three sequences, designed to maximize homology, and includes the partial C-terminal sequence of the HSV-2 140-kDa protein associated with ribonucleotide reductase (McLauchlan and Clements, 1983; Dutia, 1983; Bacchetti et al., 1984). The overall homologies, including conservative substitutions, were 37% between mouse M1 and E. coli B1 (exact matches 22%), 33% between M1 and the EBV 93-kDa reductase (exact matches 20%), and 22% between the EBV reductase and B1 (exact matches 12%). Approximately 16% of the total positions compared were conserved among all three sequences, mammalian, bacterial, and viral (exact matches 7%).

Specific regions of greater homology were found by computer as follows: M1 versus B1: residues 303-382 (homology 56%, exact matches 39%), residues 464-551 (homology 54%, exact matches 37%), and residues 1-83 (homology 52%, exact matches 33%); M1 versus EBV reductase: residues 290-371 (homology 59%, exact matches 42%); residues 501-548 (homology 65%, exact matches 42%), residues 581-639 (homology 54%, exact matches 35%), and residues 679-744 (homol-
Fig. 7. Homology between ribonucleotide reductases from mouse, E. coli, EBV, and HSV. The deduced amino acid sequences of mouse M1, E. coli B1, the EBV 93-kDa reading frame, and the partial C-terminal sequence of the HSV-2 140-kDa reductase are aligned to show maximum homology. The protein is indicated to the left of the lines with the associated number showing the position in the sequence of the first residue of that line. The numbers shown below the sequences designate positions of residues in the M1 sequence. Sequences were aligned by computer analysis combined with visual inspection. Solid bars above the sequence indicate regions of maximum homology between M1 and B1, and broken bars indicate regions of maximum homology between M1 and the EBV and HSV proteins. Asterisks show conserved cysteine residues referred to in the text. Note that both exact matches and conservative substitutions have been boxed. Details of the number of exact matches in specific regions of the sequence are given in the text. Substitutions between chemically similar residues that were allowed were: ILMVA, RKH, YFW, DE, TS, GA, QN, P, and C, where A is the only amino acid included in two groups. A, Ala; C, Cys; D, Asp; E, Ghu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.
Molecular Cloning of Mouse Ribonucleotide Reductase

We have isolated and characterized overlapping cDNAs encoding mouse ribonucleotide reductase subunit M1. The 2.9-kb composite sequence contains a single open reading frame of 2,376 nucleotides, predicting a protein of M, 90,534, which compares well with the estimated size of subunit M1 (Eriksson et al., 1981b). Definitive proof of the identity of these cDNAs was obtained by determining 15 of the first 18 residues of subunit M1 which exactly match the N-terminal sequence predicted by the cDNA (Fig. 5).

The nucleotide sequence contains a 446-base pair 3'-untranslated region with a polyadenylation signal, AATAAA (Proudfoot and Brownlee, 1976), 15 nucleotides from the poly(A) tail. Analysis of M1 mRNA on denaturing gels showed a single message of approximately 4.4 kb in S49, WEHI-7, and mouse L-cells (data not shown). The 3'-untranslated region and the coding region together cover 2822 nucleotides, leaving ~1580 nucleotides to be accounted for by the 5'-untranslated region and the poly(A) tail. Analysis of 50 clones from the second library indicated that apart from a few short clones, the majority terminated approximately 80 nucleotides upstream from the initiator AUG, suggesting that this region may contain a structural block preventing reverse transcription through to the 5' end.

Subunit M1 of ribonucleotide reductase contains three distinct nucleotide-binding sites: the catalytic site and two allosteric sites. Regulatory mutants containing alterations in each of the allosteric sites have been well-characterized (Eriksson et al., 1981a). In this study, we isolated M1 cDNA clones using mRNA from two mutant S49 cell lines, one which appears to express predominantly dATP feedback-resistant (activity site mutant) M1 and one which contains both wild-type and dGTP/dTTP-resistant (specificity site mutant) M1 (Ullman et al., 1981). A comparison of one complete sequence from each mutant line revealed no differences, suggesting that this sequence represents wild-type M1. To determine the sequence of the mutant forms of M1, we are now analyzing other cDNAs from each mutant line. This will allow us to probe the relationship of the enzyme structure to its function and lead to a deeper understanding of the molecular nature of the nucleotide binding and allosteric regulation.

There is great similarity at a functional level between ribonucleotide reductases isolated from species as widely separated as E. coli and mammals (Thelander and Reichard, 1979). The following two pieces of molecular data support this observation. Genomic DNA from a number of different species (mammalian, avian, and yeast) hybridized to mouse M1 sequences under conditions of high stringency (Fig. 7), suggesting that there is strong conservation at the DNA sequence level. We have also detected extensive amino acid sequence homology between mouse M1 and the recently published sequences of the analogous E. coli B1 (Carlson et al., 1984) and the ribonucleotide reductases of the herpesviruses, EBV (Gibson et al., 1984) and HSV (McLauchlan and Clements, 1983). Although conserved residues extend throughout the length of the polypeptide, we identified a highly homologous central domain, common to all three of the sequences compared (M1 residues 304–548, 28% conserved). Such strong sequence conservation among proteins from widely unrelated species may be taken to reflect functional constraints on the encoded product. For example, in a comparison of the amino acid sequences of bacterial and mammalian dihydrofolate reductases, approximately 70% of the conserved residues were found in the regions which form the hydrophobic binding site of the enzyme (Simonsen et al., 1989). We therefore propose that the conserved central domain in ribonucleotide reductase subunit M1 may comprise part of the catalytic site. In support of this, active dithiols have been implicated in the catalytic reduction by ribonucleotide reductase and are known to be located on subunit B1 (Thelander and Reichard, 1979). We note that this highly conserved region contains 2 conserved cysteine residues (Cys 411 and Cys 429). A third conserved cysteine (Cys 218) lies outside, but close to the region of homology. The homologies revealed two further domains, an N-terminal domain common to M1 and B1 but largely deleted in EBV, and a C-terminal domain containing sequences strongly conserved in M1 and the EBV and HSV reductases, but not in B1. Since the cellular enzymes are both subject to complex and similar allosteric regulation by deoxynucleoside triphosphates and ATP, whereas the viral enzymes are not (Langelier and Buttin, 1981; Lankinen et al., 1982), it is tempting to speculate that this N-terminal region is involved in the regulation. The significance of the C-terminal domain is presently unclear, but might reflect an interaction with a mammalian cellular component such as the second subunit of ribonucleotide reductase.

The homologies described above are consistent with the assigned map locations of the ribonucleotide reductase gene in HSV (Dutia, 1983) and EBV (Gibson et al., 1984). Our data suggest that the EBV 93-kDa reading frame and the HSV 140-kDa gene correspond to the large subunit of mammalian or E. coli ribonucleotide reductase. Although the subunit structure of the virally encoded enzymes has not been defined, an intriguing possibility is that the EBV 54-kDa reading frame (Gibson et al., 1984) and the HSV 98-kDa gene (Bacchetti et al., 1984) encode the viral equivalent of the small subunit (55 kDa) of the cellular enzyme.

The availability of an M1 cDNA should allow identification of sequences that mediate the cell cycle and/or growth regulation of this important cell enzyme. To this end, genomic sequences of mouse M1 have been isolated and are under investigation.

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