The Small Subunit of Ribonucleotide Reductase Is Encoded by One of the Most Abundant Translationally Regulated Maternal RNAs in Clam and Sea Urchin Eggs

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ABSTRACT In both clam oocytes and sea urchin eggs, fertilization triggers the synthesis of a set of proteins specified by stored maternal mRNAs. One of the most abundant of these (p41) has a molecular weight of 41,000. This paper describes the identification of p41 as the small subunit of ribonucleotide reductase, the enzyme that provides the precursors necessary for DNA synthesis. This identification is based mainly on the amino acid sequence deduced from cDNA clones corresponding to p41, which shows homology with a gene in Herpes Simplex virus that is thought to encode the small subunit of viral ribonucleotide reductase. Comparison with the B2 (small) subunit of *Escherichia coli* ribonucleotide reductase also shows striking homology in certain conserved regions of the molecule. However, our attention was originally drawn to protein p41 because it was specifically retained by an affinity column bearing the monoclonal antibody YL 1/2, which reacts with α-tubulin (Kilmartin, J. V., B. Wright, and C. Milstein, 1982, *J. Cell Biol.*, 93:576–582). The finding that this antibody inhibits the activity of sea urchin embryo ribonucleotide reductase confirmed the identity of p41 as the small subunit. The unexpected binding of the small subunit of ribonucleotide reductase can be accounted for by its carboxy-terminal sequence, which matches the specificity requirements of YL 1/2 as determined by Wehland et al. (Wehland, J., H. C. Schroeder, and K. Weber, 1984, *EMBO [Eur. Mol. Biol. Organ.] J.*, 3:1295–1300).

Unlike the small subunit, there is no sign of synthesis of a corresponding large subunit of ribonucleotide reductase after fertilization. Since most enzymes of this type require two subunits for activity, we suspect that the unfertilized oocytes contain a stockpile of large subunits ready for combination with newly made small subunits. Thus, synthesis of the small subunit of ribonucleotide reductase represents a very clear example of the developmental regulation of enzyme activity by control of gene expression at the level of translation.

Most of the structural proteins and enzymes required to support the rapid cleavage divisions of the early embryo are already present in full-grown oocytes and unfertilized eggs. Well-studied examples include DNA polymerase, chromosomal proteins, ribosomes, actin, and tubulin (21, 31, 33, 38, 40, 41). However, in addition to proteins stored as such, these cells also contain a store of "masked" maternal mRNA which is not translated to a significant extent (if at all) until after fertilization (reviewed in references 7 and 42). Many of the polypeptides encoded by these mRNAs do not correspond to those already present in the egg. These messages thus represent a potential future stock of proteins that can be produced rapidly after fertilization without requiring the synthesis of new RNA. The fact that these proteins are stored in "virtual" form as mRNA, rather than as the actual proteins themselves suggests that it may not be desirable or appropriate for the egg to contain this class of protein. For example, the egg does not undergo mitosis until after fertilization, and new protein

THE JOURNAL OF CELL BIOLOGY · VOLUME 100 June 1985 1968–1976

synthesis is absolutely necessary after fertilization for the very first cleavage division (25, 53, 55, 58, 59). This strongly suggests that maternal mRNA must specify one or more proteins that are essential catalysts of cell division (58). We have previously described how one (in the sea urchin *Arbacia punctulata*) or two (in the clam *Spisula solidissima*) of the abundant maternal mRNAs encode proteins that begin to be synthesized very shortly after fertilization, and that are very specifically and suddenly destroyed at particular points in the cell cycle as though they were involved in some aspect of the regulation of progress through the cell cycle (20).

This paper describes the identification of a maternal mRNA that encodes a polypeptide of 41,000 mol wt, whose synthesis is strongly activated shortly after fertilization. It appears to be one of the most abundant of all the mRNAs stored in both clam oocytes and sea urchin eggs. We show here that it is the small subunit of the enzyme ribonucleotide reductase. This is not altogether surprising, since ribonucleotide reductase is an essential enzyme for DNA replication, and the rate of DNA synthesis in early embryos rises to very high levels before slackening off after a few hours. This enzyme converts ribonucleotides to deoxyribonucleotides and thus provides the precursors for DNA synthesis (reviewed in references 24 and 51). In sea urchin eggs the pool of dNTPs is sufficient for only about two rounds of DNA synthesis after fertilization, so ribonucleotide reductase activity is required for further development (6, 12, 34, 37). The requirement for newly synthesized deoxyribonucleotides is clearly shown by the arrest of cell division by hydroxyurea, a specific inhibitor of ribonucleotide reductase. Hydroxyurea arrests the development of sea urchin embryos at the four-cell stage (6) and prevents completion of even the first round of DNA synthesis in clams (our unpublished observations). It has been known for some time that unfertilized sea urchin eggs have very low or undetectable levels of ribonucleotide reductase activity and that the appearance of enzyme activity requires protein synthesis but not new transcription (37). These are exactly the characteristics expected of an enzyme encoded by a stored maternal mRNA whose translation is initiated after fertilization. Until now it was not formally possible to rule out an alternative model in which protein synthesis provided an activator of a preexisting latent form of the enzyme. The identification of a very abundant mRNA for the small subunit of ribonucleotide reductase seems to rule out this hypothesis, although, as we point out in the Discussion, we would expect to find a mRNA encoding the other (large) subunit of the enzyme, and there is no sign of such a species.

MATERIALS AND METHODS

Buffers: We used the following buffers: buffer A, 300 mM glycine, 120 mM glutamic acid, 100 mM HEPES, 100 mM taurine, 40 mM NaCl, 10 mM EGTA, 2.5 mM $MgCl_2$, adjusted to pH 7.2 with KOH; buffer B, 20 mM HEPES, 10 mM $MgCl_2$, 2 mM dithiothreitol, adjusted to pH 7.6 with KOH; buffer C, 50 mM Tris-Cl, 100 mM KCl, pH 7.6; buffer D, 700 mM 2-mercaptoethanol, 125 mM Tris-Cl, 5% vol/vol glycerol, 2% wt/vol SDS, 0.0015% wt/vol bromophenol blue, pH 6.8; and calcium-free seawater, 0.38 M NaCl, 50 mM $MgCl_2$, 30 mM Na_2SO_4, 20 mM HEPES, 10 mM KCl, 2 mM $NaHCO_3$, 1 mM EGTA, pH 7.4, with NaOH.

Animals: Clams (*Spisula solidissima*) and sea urchins (*Arbacia punctulata*) were obtained from the Department of Marine Resources, Marine Biological Laboratory, Woods Hole, MA). They were kept in running sea water, the clams at 13–15°C, the urchins at 18–20°C. Gametes were isolated and cultured as previously described (20, 44).

Enzymes and Radioisotopes: Restriction enzymes, T4 DNA ligase, and the large subunit of *Escherichia coli* DNA polymerase I (Klenow fragment) were purchased from New England Biolabs (Beverly, MA). Sequencing primers were obtained either from Collaborative Research Inc. (Lexington, MA) or New England Biolabs.

[^{35}S]methionine (>1,000 Ci/mM) and γ-[^{32}P]ATP (>3,000 Ci/mM) were purchased from Amersham Corp. (Arlington Heights, IL) and α-[^{32}P]dATP and [^{35}S]dATP-α-S from New England Nuclear (Boston, MA).

Rat anti-yeast α-tubulin monoclonal antibody YL 1/2 coupled to cyanogen bromide–activated Sepharose CL-4B was a gift of Dr. J. Kilmartin, MRC laboratory for Molecular Biology, Cambridge, England. Other antibodies were the gift of Dr. L. Thelander, Karolinska Institute, Stockholm.

Taxol was obtained from the Natural Products Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD).

Acrylamide Gel Electrophoresis: Acrylamide gels were poured and run essentially as described by Laemmli (32) and modified by Anderson et al. (3). The only further modification (suggested by Dr. A. Murray, Harvard University) was to omit SDS from both stacking and resolving gels so that stock solutions, requiring only the addition of the polymerization catalysts, could be stored at 4°C. This procedure is much more convenient and gives somewhat more reproducible results with no detectable loss of resolution. We used slabs ~140 × 80 × 0.8 mm with a stacking gel ~5 mm high. They were run at 150 V until the tracking dye ran off the bottom.

Immunoaffinity Chromatography: 3 ml of a suspension of fertilized *Arbacia* eggs (15,000–20,000/ml) was incubated in filtered (0.45-μm pore filters; Millipore Corp., Bedford, MA) seawater at 20°C. [^{35}S]methionine (25 μCi/ml) was added 5 min after fertilization, once it was clear that >90% of the eggs had raised a fertilization envelope. The incubation was continued for 3 h with occasional gentle swirling. The embryos were collected using a hand centrifuge, and washed twice with 5 ml ice-cold Ca^{2+}-free seawater and once with buffer A. They were homogenized in 200 μl of buffer A containing 1 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride (from a 0.1 M stock in dimethylsulfoxide) with five strokes of a 5-ml stainless steel Dounce homogenizer (Kontes Glass Co., Vineland, NJ). The homogenate was centrifuged for 10 min at 10,000 rpm (12,000 *g*) at 4°C in the Sorvall SS34 rotor (DuPont Instruments—Sorvall Biomedical Div., DuPont Co., Wilmington, DE), and the supernatant was stored in 40-μl aliquots in liquid nitrogen.

The extracts were thawed and applied to 100 μl columns of Sepharose CL-4B or CL-4B coupled to the monoclonal antibody YL 1/2 (29) contained in 200-μl yellow Eppendorf pipette tips plugged with glass wool and previously equilibrated with buffer A. The entire 40-μl sample was loaded, then washed with 100 μl or buffer A to yield a flow-through fraction. After a wash with 500 μl of buffer A, the columns were eluted with buffer D. Samples (2.5 μl) of the load and flow-through fractions were diluted five-fold with buffer D for analysis on acrylamide gels, whereas the fractions eluted with buffer D were loaded directly.

In Vitro Assembly of Microtubules with Taxol: The method of Vallee (54) was followed to make taxol precipitates of microtubules. Clam oocytes were activated by the addition of 40 mM excess KCl to a suspension of ~20,000 cells/ml in filtered seawater. Typically, 10 μl of 10 mCi/ml [^{35}S] methionine was added to 3 ml of suspension and incubated for 70 min at 20°C. A parallel culture without label served as carrier. The oocytes were harvested, washed, homogenized, and clarified as described above, but instead of the 12,000 *g* supernatants' being frozen, they were immediately centrifuged at 180,000 *g* in the Beckman Ti70 rotor at 4°C (Beckman Instruments Inc., Palo Alto, CA) for 60 min. The resulting post-ribosomal supernatants (containing cold-soluble tubulin) were made 4 mM in Mg^{2+}–GTP and divided into two portions. One portion was made 40 μM in taxol (from a stock in dimethylsulfoxide), and the other had an equivalent amount of dimethylsulfoxide added. They were incubated at 20°C (physiological temperature for clams; mammalian cell extracts need 37°C at this step) for 15 min. The extracts were chilled on ice and carefully layered over 5% sucrose cushions in buffer A containing either 4 mM Mg^{2+}–GTP or 4 mM Mg^{2+}–GTP plus 40 μM taxol as appropriate. They were centrifuged for 25 min at 180,000 *g* at 4°C. A cloudy pellet was visible in the taxol sample, whereas no pellet could be seen in the control. Samples (100 μl) of the supernatants were analyzed on YL 1/2 immunoaffinity columns, and the pellets were dissolved directly in buffer D.

Ribonucleotide Reductase Assays: *Arbacia punctulata* eggs were fertilized and allowed to develop for 7–8 h at 20°C. The blastulae were washed twice in Ca^{2+}-free seawater and frozen as cell pellets in liquid nitrogen. The pellets were later suspended in 3 vol buffer B and homogenized with several strokes of the Dounce homogenizer until cell lysis was essentially complete, as judged by microscopic examination. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant was made 2 M in $(NH_4)_2SO_4$ by addition of an equal volume of saturated solution. After 30 min on ice, the pellet was collected by centrifugation at 10,000 rpm for 15 min, dissolved in a minimum volume of buffer C to give a protein concentration of ~10 mg/ml, and desalted by passage through a small column of Sephadex G50 equilibrated

with buffer C. Fractions containing 2–5 mg/ml protein were pooled and assayed for ribonucleotide reductase activity by the reduction of [^{3}H]CDP to dCDP as described by Engström et al. (18). These assays were carried out in the laboratory of Dr. L. Thelander, to whom we are most grateful.

DNA Sequencing: The isolation of cDNA clone 1T43 complementary to clam p41 mRNA was described by Rosenthal et al. (45). Two other clones, B11 and 2 (Rosenthal, E. T. and Ruderman, J. V., unpublished observation) were identified by hybrid-selected translation (43). The cDNA inserts were cut out of the plasmid DNA with restriction enzyme Pst I, purified by agarose gel electrophoresis, electroeluted from the gel slice, further digested with suitable restriction enzymes, and inserted into the appropriately linearized replicative form of M13 mp8 or mp9 cloning vehicles (36). Restriction fragments containing the insert-flanking G–C tails and Pst I site were subcloned in only one orientation, with the Pst I site distal to the sequencing primer site. Single-stranded DNA templates were prepared and sequenced by the dideoxy chain termination method (5, 46).

The cDNA clone corresponding to mRNA for sea urchin p41 was obtained as follows (8). Double stranded cDNA was prepared from egg poly(A)$^+$ mRNA according to standard procedures (16). The cDNA was then digested to completion with restriction enzyme Sau 3A and ligated into the Bam HI site of M13 mp8. Recombinant phage were screened with egg poly(A)$^+$ labeled with polynucleotide kinase and γ-[^{32}P]ATP; this identified clones complementary in sequence to abundant mRNAs. These were grown up and tested by hybrid-selected translation. Clone A18 2A strongly selected an mRNA encoding p41, and was sequenced as described above.

RESULTS

p41 Binds to a Monoclonal Anti-α-Tubulin Antibody Affinity Column

Both clam oocytes and sea urchin eggs contain large amounts of a stored maternal mRNA encoding a 41,000 mol wt protein whose translation is strongly activated after fertilization (20, 44, 45). These proteins, previously called "protein C" in the clam and "protein B" in the urchin will be referred to hereafter as p41. Evans et al. (20) made the observation that sea urchin p41 bound to an affinity resin carrying the rat anti-yeast α-tubulin antibody YL 1/2 isolated by Kilmartin et al. (29), during the course of experiments testing for tubulin synthesis in early sea urchin blastulae. None of the other newly synthesized proteins showed detectable binding to this affinity column, though the unlabeled tubulin present in the extracts appeared to be quantitatively retained, as expected.

Fig. 1 shows the binding of urchin p41 that occurred when an extract of [^{35}S]methionine-labeled sea urchin embryos was passed over Sepharose 4B coupled to YL 1/2. Fig. 1, lane *F* shows the autoradiogram of the load, with p41 visible as the strongest band in the middle of the gel, indicated by an arrow and the label RR. Fig. 1, lane *G* is the flow-through of the column, from which p41 is almost completely removed. Fig. 1, lanes *H–J*, shows the autoradiogram of the eluate obtained by washing the column with SDS gel sample buffer (buffer *D*). The positions of stained, α- and β-tubulin from chick brain are indicated in Fig. 1, lane *K*. Fig. 1, lanes *L–N*, displays the equivalent stained lanes of load, flow-through, and bound material respectively, which shows that the column is very specific for tubulin. Apart from the tubulins, the only other visible stained band in the eluate is the light chain of the antibody (which is dissociated from the heavy chain by the SDS gel sample buffer). It is interesting that no radioactivity in tubulin was detected in these experiments, although its synthesis has previously been detected in other species of sea urchin (41). However, the rate of tubulin synthesis is very low at this early stage (1, 2), and there may have been too little incorporation of label to detect by the exposures shown here. It is however also possible that the newly synthesized protein lacks the carboxy-terminal tyrosine residue necessary for binding to this particular antibody (57). This point might warrant further study.

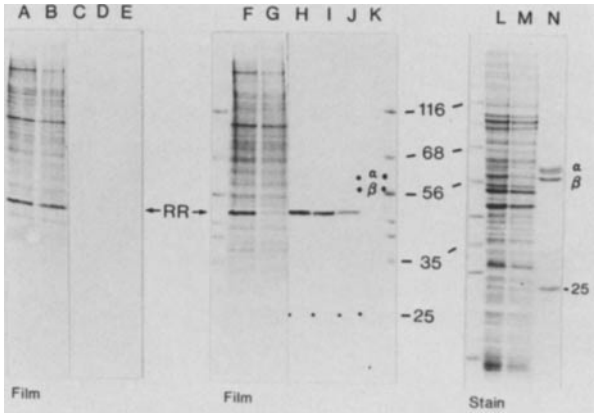

FIGURE 1 The monoclonal antibody YL 1/2 binds newly synthesized p41 from sea urchin embryos, as well as tubulin. An [^{35}S]-methionine-labeled homogenate of sea urchin embryos was fractionated on either unmodified Sepharose 4B (*left*) or Sepharose 4B coupled to YL 1/2 antibody (*middle* and *right*). Samples were analyzed on 15% SDS plyacrylamide gels. Lanes *A–K*, autoradiograms; lanes *L–N*, Coomassie stain. Lane *A*, column load; lane *B*, flow-through; lanes *C–E*, bound fraction eluted from a control column of unsubstituted Sepharose 4B. Lane *F*, load; lane *G* flow-through; lanes *H–J*, bound fractions eluted from the YL 1/2 affinity column. Lane *K*, position of chick brain α- and β-tubulins. Lanes *L–N*, load, flow-through, and bound fractions analyzed by Coomassie Blue stain, respectively. The positions of the sea urchin tubulins and the light chain of the antibody are indicated. *RR*, position of p41.

The binding of p41 to this column seemed to require antibody YL 1/2, since unsubstituted Sepharose did not retain it; lanes *A–E* of Fig. 1 are directly comparable to lanes *F–J*, except for the absence of matrix-bound antibody. We also tested Sepharose coupled to a different antibody, and did not observe any binding of radioactive proteins (data not shown).

We next tested similar extracts of clam embryos and found that clam p41 also showed this high-affinity, high-specificity binding to the YL 1/2 Sepharose. This can be seen in Fig. 2, lanes *H*, *L*, and *M*. Considering that clam and urchin p41 had very similar mobility on SDS polyacrylamide gels, that neither of them was synthesized to a significant extent before fertilization though they both became two of the most strongly labeled bands soon after, and that they both showed unexpected binding to antibody YL 1/2, we thought it likely that they were homologous proteins. It was clear that neither of them was tubulin, for not only were they much too small, but neither of them precipitated with vinblastine under conditions where tubulin paracrystals were efficiently formed (data not shown). However, since they bound avidly to the YL 1/2 affinity column and the extracts passed over the columns contained high levels of soluble tubulin, we thought it most likely that the binding was indirect and occurred via tubulin—in other words, that p41 might be a tubulin-associated protein, perhaps involved in the assembly of the mitotic spindles during cleavage. Note that β-tubulin does not bind to YL 1/2 at all by itself, but in association with α-tubulin it is quantitatively retained (29).

To test this idea we exploited the properties of taxol, a drug that causes the GTP-dependent polymerization of tubulin and microtubule-associated proteins into cold-stable micro-

tubules that can be recovered by high-speed centrifugation (47). We performed an experiment with labeled clam embryos to see if p41 co-sedimented with the taxol-stabilized microtubules and, if not, whether the p41 left in the supernatant would still bind to the antibody affinity column in the absence of tubulin. The experiment is described in Materials and Methods and shown in Fig. 2. A large pellet of microtubules was formed in the presence of taxol (Fig. 2, lane *B*), but no labeled p41 was present (Fig. 2, lane *F*). This seemed to rule out the possibility that p41 was a microtubule-associated protein. Next, the 180,000 *g* supernatants were passed over the YL 1/2 affinity column; the bound fractions are shown in Fig. 2, lanes *I* and *J* by Coomassie Blue stain, and in lanes *L* and *M* by autoradiography. The control supernatant (i.e., without taxol) clearly contains tubulin (Fig. 2, lane *I*), whereas the taxol supernatant is almost completely devoid of it (lane *J*). However, labeled p41 was retained with equal strength in both cases (compare Fig. 2, lanes *L* and *M*). Thus p41 appears to have an intrinsic direct affinity for the antitubulin antibody YL 1/2 and does not bind via tubulin as we had at first speculated. The probable explanation for this binding is found by examination of its carboxy-terminal amino acid sequence, which matches the recognition specificity of the antibody, recently determined by Wehland et al. (56), as discussed later.

Nucleotide Sequence of Clam p41 mRNA

Since cDNA clones were already available for both clam and urchin p41 mRNAs (8, 45), we decided to determine their nucleotide sequences in order to see if they showed homology with each other and with any other known proteins. Fig. 3 shows the sequencing strategy for the series of overlapping cDNA clones corresponding to clam p41. A sequence of 1,334 nucleotides and the deduced amino acid sequence are shown in Fig. 4. The mRNA is ~1,700 nucleotides long (45), so neither the 5′ nor the 3′ noncoding sequences shown here are complete. The only open reading frame of significant length contains 300 codons and extends from the third AUG at position 299 to a UAA codon at position 1199. The molecular weight of the corresponding polypeptide is 32,787, significantly less than its apparent size of 41,000 derived from SDS polyacrylamide gels. Since most, though not all, eucaryotic mRNAs use the first AUG to initiate translation (30) and this discrepancy exists between the observed and predicted size of p41, there may be a frameshift error between positions 123 and 298, either in clone B11 itself or in its sequence determination. If the first AUG at position 120 were the true initiation codon, the predicted polypeptide would have a molecular weight of ~39,000. We are in the process of directly determining the amino-terminal amino acid sequence of clam p41 in order to find where translation is initiated.

Amino Acid Sequences of Clam and Sea Urchin p41s Show Homology

Inspection of a 49 residue amino acid sequence of sea urchin p41, deduced from a 147-base cDNA clone constructed and sequenced as described in Materials and Methods revealed a strong homology with a corresponding stretch of clam p41 starting at position 518. This is shown in Fig. 5; of 49 amino acids compared, 34 are identical and 5 more are highly conservative substitutions. This extremely high homology is not so well preserved at the nucleotide level. Two-thirds of the identical amino acids are specified by different

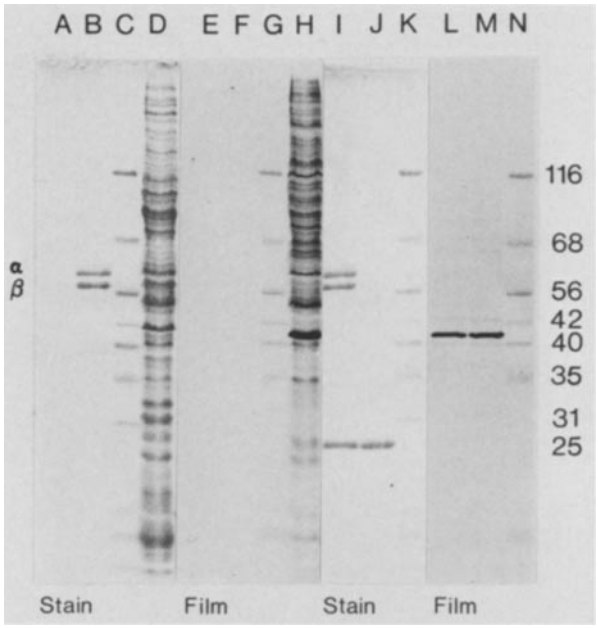

FIGURE 2 Clam p41 does not bind to taxol-stabilized microtubules and does not require tubulin to bind to antibody YL 1/2. Clam oocytes were activated with KCl and labeled with [^{35}S]methionine for 70 min. A post-ribosomal supernatant prepared at 4°C to disaggregate microtubules was incubated with or without taxol in the presence of Mg^{2+}–GTP at 20°C for 15 min. The samples were chilled and centrifuged again at 180,000 *g*. Lane *A*, Coomassie Blue stain of the resuspended pellet from the control tube; lane *B*, the same fraction from the taxol sample. Lanes *C*, *G*, *K*, and *N*, molecular weight standards. Lane *D*, starting post-ribosomal supernatant. Lanes *E–H*, autoradiograms of lanes *A–D*. Lane *I*, bound fraction eluted from YL 1/2 Sepharose loaded with the control 180,000 *g* supernatant, lane *J*, equivalent sample from a column loaded with the taxol 180,000 *g* supernatant. Lanes *L* and *M*, the corresponding autoradiographs.

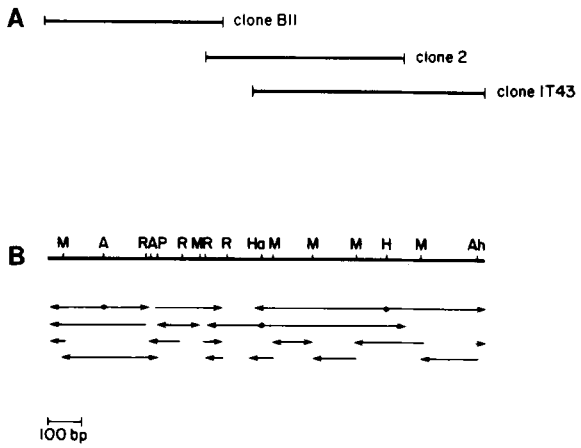

FIGURE 3 Sequencing strategy for an overlapping set of cDNA clones corresponding to clam p41 mRNA. Clones B11, 2, and 1T43 represent dC-tailed cDNA sequences inserted into the Pst I site of pBR322. The extent of overlap of the clones is shown in *A*. Inserts were excised with Pst I, and the restriction fragments indicated in *B* were subcloned into M13 vectors. The restriction enzyme cleavage sites used are Alu I (*A*); Aha III (*Ah*); Hae III (*Ha*); Hind III (*H*); Mnl I (*M*); Pvu II (*P*); and Rsa I (*R*). The lengths and directions of the sequences read are indicated by the arrows. The map is oriented with the 5′ end of the mRNA to the left. *bp*, base pairs.

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                10         20         30         40         50         60
.....TTGTTATTTC TCCAAGGACT TTCTTTGGCT TATTTTCCTC GTAATCTTCT ATTTTCCGAG

                70         80         90        100        110        120
        GTTTCCTGAC AATTCGTTTT CCTTTCGGGT TGTATTTATT GAAAGCATTG TCGTTCAGTA

               130        140        150        160        170        180
        TGAAATTTGT AAGTAACAGT TTCAAAATTA CGTTTTTCAG AAAAAGTTTA GCTACTTGAC

               190        200        210        220        230        240
        AGAACGAATA TCAAAAGTTA AACCTGTAGT AAAGAAGTCT CAACAAGTAG AACCATTATT

               250        260        270        280        290
        GGCTGACAAC CCTAGACGTT TTGTTGTACT ACCTATACAA TACCATGATA TCTGGAAG

300                        320                        340
 Met Tyr Lys Lys Ala Glu Ala Ser Phe Trp Thr Ala Glu Glu Val Asp Leu Ser Lys Asp
 ATG TAC AAA AAA GCT GAG GCA TCA TTC TGG ACA GCT GAG GAA GTG GAC CTT TCA AAA GAC

360                        380                        400
 Met Ala His Trp Glu Ser Leu Lys Lys Glu Glu Lys His Phe Ile Ser His Val Leu Ala
 ATG GCA CAC TGG GAA TCC CTG AAG AAA GAA GAG AAA CAT TTC ATC TCA CAT GTA CTT GCT

420                        440                        460
 Phe Phe Ala Ala Ser Asp Gly Ile Val Asn Glu Asn Leu Val Glu Arg Phe Ser Lys Glu
 TTC TTT GCT GCC AGT GAT GGA ATT GTA AAT GAA AAC CTG GTT GAA AGA TTC AGT AAG GAG

480                        500                        520
 Val Gln Val Thr Glu Ala Arg Cys Phe Tyr Gly Phe Gln Ile Ala Met Glu Asn Ile His
 GTA CAG GTG ACT GAG GCA AGA TGT TTC TAT GGT TTC CAA ATT GCA ATG GAG AAC ATT CAT

540                        560                        580
 Ser Glu Met Tyr Ser Leu Leu Ile Asp Thr Tyr Ile Lys Asp Pro Gln Glu Arg Asp Phe
 TCA GAA ATG TAC AGT TTA CTC ATA GAT ACT TAC ATC AAA GAT CCA CAA GAA AGG GAT TTC

600                        620                        640
 Leu Phe Asn Ala Ile Glu Thr Met Pro Cys Val Lys Glu Lys Ala Asp Trp Ala Met Arg
 CTA TTT AAT GCA ATT GAG ACT ATG CCA TGT GTA AAG GAA AAG GCT GAC TGG GCC ATG CGT

660                        680                        700
 Trp Ile Asn Asp Asp Ser Ser Ser Tyr Ala Glu Arg Val Val Ala Phe Ala Ala Val Glu
 TGG ATC AAC GAT GAT TCC TCA TCA TAT GCT GAA CGA GTT GTT GCA TTT GCT GCT GTT GAA

720                        740                        760
 Gly Ile Phe Phe Ser Gly Ser Phe Ala Ser Ile Phe Trp Leu Lys Lys Arg Gly Ile Met
 GGA ATT TTC TTC TCA GGA TCA TTT GCT TCA ATA TTT TGG TTG AAG AAA CGT GGA ATC ATG

780                        800                        820
 Pro Gly Leu Thr Phe Ser Asn Glu Leu Ile Ser Arg Asp Glu Gly Leu His Cys Asp Phe
 CCA GGT CTT ACA TTC AGT AAT GAA CTG ATC AGC AGA GAT GAG GGT CTT CAC TGT GAT TTT

840                        860                        880
 Ala Cys Leu Met Phe Ser His Leu Val Asn Lys Pro Ser Gln Glu Arg Ile His Gln Ile
 GCT TGT CTT ATG TTT AGC CAT CTT GTA AAT AAA CCA TCT CAA GAA AGA ATA CAT CAA ATC

900                        920                        940
 Ile Asp Glu Ala Val Lys Ile Glu Gln Val Phe Leu Thr Glu Ala Leu Pro Cys Arg Leu
 ATA GAT GAA GCT GTT AAA ATT GAA CAA GTA TTC CTC ACT GAA GCA CTC CCA TGC AGA CTT

960                        980                       1000
 Ile Gly Met Asn Cys Asp Leu Met Arg Gln Tyr Ile Glu Phe Val Ala Asp Arg Leu Leu
 ATT GGA ATG AAC TGT GAT CTT ATG AGA CAA TAC ATT GAG TTT GTT GCC GAC AGA TTG CTG

1020                       1040                       1060
  Leu Glu Leu Lys Cys Asp Lys Leu Tyr Asn Lys Glu Asn Pro Phe Asp Phe Met Glu His
  TTA GAA TTA AAA TGT GAT AAG CTT TAC AAC AAA GAA AAT CCC TTT GAC TTT ATG GAG CAT

1080                       1100                       1120
  Ile Ser Leu Glu Gly Lys Thr Asn Phe Phe Glu Lys Arg Val Gly Glu Tyr Gln Lys Met
  ATA TCA TTG GAA GGA AAA ACT AAC TTT TTT GAA AAA CGT GTT GGT GAA TAT CAG AAG ATG

1140                       1160                       1180
  Gly Val Met Ser Gly Gly Asn Thr Gly Asp Ser His Ala Phe Thr Leu Asp Ala Asp Phe
  GGC GTT ATG TCA GGA GGA AAC ACA GGG GAC AGT CAT GCA TTT ACC TTA GAT GCT GAT TTC

     1200       1210       1220       1230       1240       1250       1260
       TA AACTACTGAA AAACCATGTG AAAGTGATTG CTCATTAGAT TTGTGTAAGA ATCCAAGAAC

            1270       1280       1290       1300       1310       1320
         TTTTTTGTGG TTCATCGACA ATACTGGAAG CGTTGTTGTT TCATTGGTTT TAAAACATTT

            1330
         TAATAATAGT GCAA.....................................................
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FIGURE 4 The nucleotide sequence of clam p41 mRNA and the amino acid sequence of p41 derived from it. The combined sequences of the three overlapping cDNA clones shown in Fig. 1 were translated in all six reading frames; the 300 amino acid sequence shown here represents the longest open reading frame, starting at the third AUG at position 299. Upstream out-of-frame AUGs, which occur at positions 120 and 285, are underlined. Additional sequences of the full-length 1,700 nucleotide mRNA remain to be determined at both ends, indicated by dotted lines. The final digits of the numbers are aligned with the nucleotide positions to which they refer.

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Clam
ATT GCA ATG GAG AAC ATT CAT TCA GAA ATG TAC AGT TTA CTC ATA GAT ACT TAC ATC AAA
**C **C **C *** *** **C **C **T **G *** *** TC* C*G *** **C **C **C GTA C** **G
Ile Ala Met Glu Asn Ile His Ser Glu Met Tyr Ser Leu Leu Ile Asp Thr Tyr Ile Lys
 *   *  Ile  *   *   *   *   *   *   *   *   *   *   *   *   *  Val Leu  *
Sea urchin

Clam
GAT CCA CAA GAA AGG GAT TTC CTA TTT AAT GCA ATT GAG ACT ATG CCA TGT GTA AAG GAA
*** G*C A*G C*G **A **C *** **G **C **C **T G** *** **G C*T *** *** **G GCC A*G
Asp Pro Gln Glu Arg Asp Phe Leu Phe Asn Ala Ile Glu Thr Met Pro Cys Val Lys Glu
 *  Ala Lys Gln  *   *   *   *   *   *   *  Val  *   *  Leu  *   *   *  Ala Lys
Sea urchin

Clam
AAG GCT GAC TGG GCC ATG CGT TGG ATC
**A *** C*G *** **A C*T G*A CCT GGA
Lys Ala Asp Trp Ala Met Arg Trp Ile
 *   *  Gln  *   *  Leu Gly Pro Gly
Sea urchin
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FIGURE 5 Nucleotide and amino acid homologies between clam and sea urchin p41. The nucleotide sequence of mRNA for clam p41 (*top line*) is compared with the corresponding sequence derived from a short cDNA clone complementary to sea urchin p41 mRNA. The amino acid sequences are shown below. Residues that are the same in both sequences are indicated by asterisks.

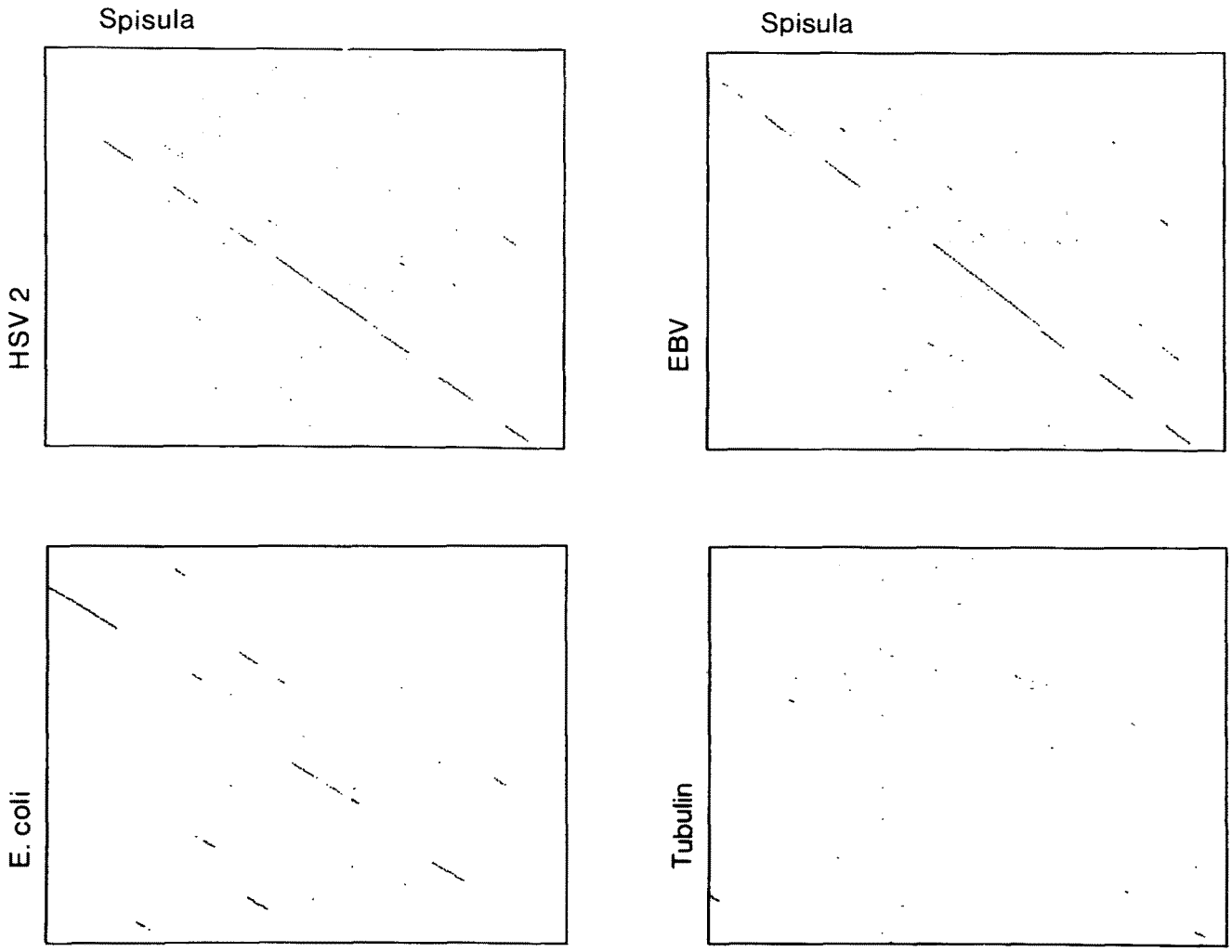

FIGURE 6 DIAGON plots of homologies between clam p41, HSV-2 p38, EBV p34, *E. coli* B2 subunit of ribonucleotide reductase, and pig α-tubulin. Clam p41 (horizontal axis) was compared with the four sequences (vertical axes) by the DIAGON program of Staden (49), using a span length of 21 and a minimum score for display of 235. The tubulin plot is included to show how a completely unrelated protein scores at this level of stringency. The Herpes Simplex virus sequence is from McLaughlan and Clements (35), EBV from Gibson et al. (23), *E. coli* from Carlson et al. (9), and tubulin from Ponstingl et al. (39).

codons, which mostly differ in the third base. This degree of mismatch easily accounts for our failure to observe cross-hybridization of the two sequences under standard conditions (data not shown).

Clam p41 Shows Homology to the Small Subunit of Ribonucleotide Reductase

When the complete amino acid sequence of clam p41 was compared with the Newat databank compiled by Doolittle (13), the surprising result was a strong resemblance to a 39,000-mol-wt Herpes Simplex virus-1 protein, whose partial amino acid sequence had been inferred from its DNA sequence by Draper et al. (14). Fig. 6 compares the predicted amino acid sequence of clam p41 with that of the corresponding gene in Herpes Simplex virus-2 (HSV-2),[1] which codes for

[1] *Abbreviations used in this paper:* EBV, Epstein-Barr virus; HSV-2, Herpes Simplex virus-2.

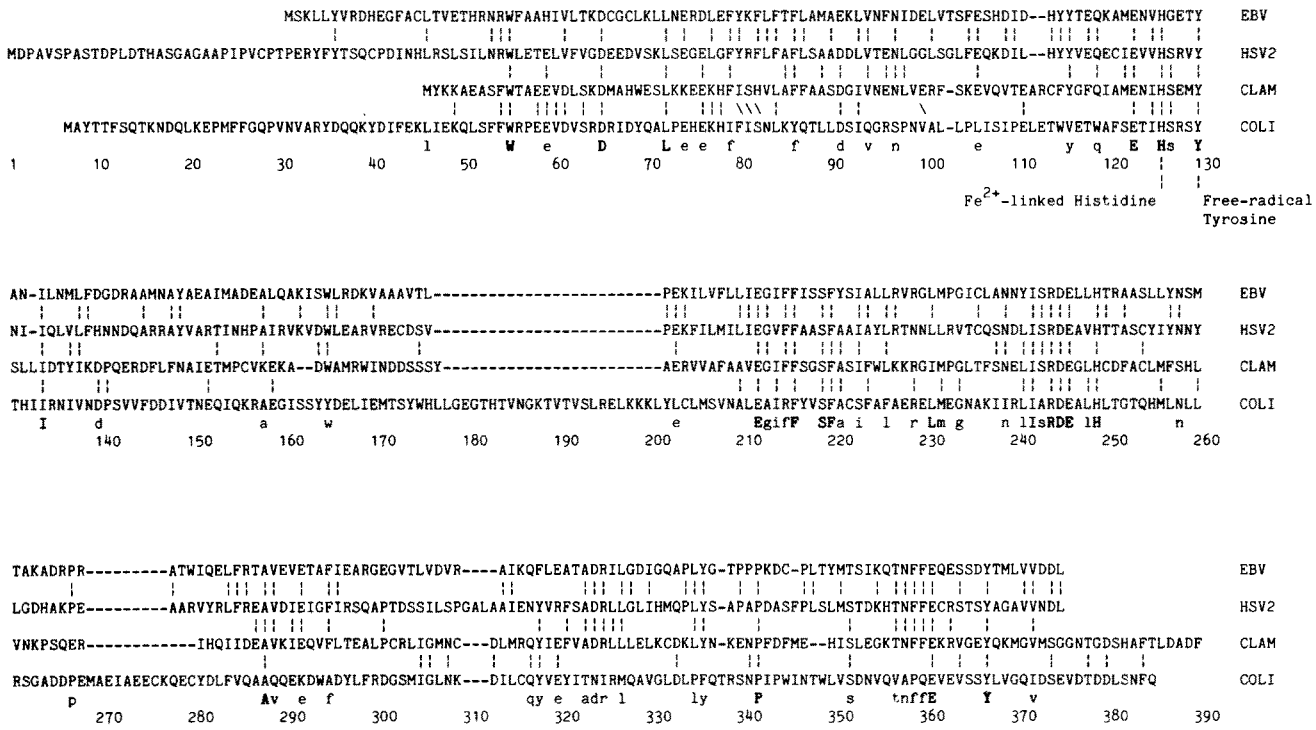

FIGURE 7 Homologies between the small subunits of ribonucleotide reductase from HSV-2, EBV, clam (*Spisula solidissima*), and *E. coli*. The sequences shown here were aligned by eye, based on the DIAGON plots shown in Fig. 6. Gaps (---) were introduced to maximize homologies. Capital letters on the bottom line indicate an amino acid that occurs at that position in all four sequences; lowercase letters represent a residue common to three of four. Conservative substitutions, which score quite well on the DIAGON matrix, are not indicated. The numbers represent the arbitrary positions referred to in the text. This alignment produces 66 exact matches between clam and *E. coli*, 76 between clam and Herpes Simplex virus, 79 between clam and EBV, and 112 between the two viral sequences. Thus homology between clam and the others is 20–25%.

a 38,000-mol-wt protein (35), using Staden's DIAGON program (49). A similar homology is seen between clam p41 and a 34,000-mol-wt protein from Epstein-Barr virus (EBV) which corresponds to HSV-2 p38 (23).

At the time that we discovered the homology between clam p41 and HSV-2 p38, the function of the viral polypeptide was not known for certain, although there was strong indirect evidence that it was involved in ribonucleotide reductase activity. Herpes virus–infected cells contain a ribonucleotide reductase activity distinct from the host cell's enzyme (10, 15, 26). Partial purification of the virus-associated enzyme suggested that it contained a 140,000-mol-wt polypeptide associated with smaller components including p38 (28). A temperature sensitive mutation in ribonucleotide reductase activity was mapped to the p140 gene (14) which is adjacent to the p38 gene and expressed at the same early time after viral infection. The two polypeptides tend to co-precipitate with antibodies raised against viral reductase (4, 22, 26–28). In almost all organisms studied so far, ribonucleotide reductase is composed of two nonidentical subunits, a smaller one of ~40,000 mol wt and a larger one of 80,000–90,000 mol wt (9, 51). It thus seemed extremely likely that the homologous proteins Herpes Simplex virus-1 p39, HSV-2 p38, EBV p34, clam p41, and urchin p41 were the small subunits of ribonucleotide reductase.

The complete amino acid sequence of *E. coli* ribonucleotide reductase was recently determined by Carlson et al. (9), and the DIAGON comparison between clam p41 and *E. coli* B2 subunit of ribonucleotide reductase shows three stretches of good homology. The plot of clam p41 against pig α-tubulin is shown as a negative control for comparison. Fig. 7 shows a manual alignment of all four homologous polypeptide sequences: EBV p34, HSV-2 p38, clam p41, and *E. coli* B2 subunit. Gaps have been introduced to maximize the homology between them. Three major clusters of strong homology are seen, at positions 123–134, 210–221, and 241–249. The tyrosine residue at position 130 in the first of these clusters probably carries the free radical involved in the catalytic mechanism, and the conserved histidine at position 126 is likely to be liganded to the iron center necessary for activity (48). Outside of these clusters, only 7 amino acids are conserved among all four sequences, though an additional 41 amino acids are shared by three of the four, and regions of weaker homology (i.e., conservative substitutions) are scattered throughout the molecule. These show up clearly in the DIAGON plots.

Although these homologies between clam p41, the viral polypeptides, and the B2 subunit of *E. coli* ribonucleotide reductase are highly suggestive, we do not feel that they constitute absolute proof of identity, particularly since the only member of the set to have been characterized by its enzymic activity is the *E. coli* enzyme, which shows the weakest homology with all the other sequences. The best evidence would be isolation of pure p41 and demonstration of its activity in vitro. Although we have not yet attempted such purification, we have found that incubating extracts of *Arbacia* 7–8-h blastulae with the YL 1/2 antibody inhibits their ribonucleotide reductase activity (Table I). The concentration of antibody required for complete inhibition was quite high, but two other antibodies did not show significant inhi-

TABLE I

Inhibition of Sea Urchin Ribonucleotide Reductase Activity by Monoclonal Antibody YL 1/2

Addition	Concentration of antibody	Activity remaining
	mg/ml	*%*
None	—	100.0
YOL 1/34	0.98	99.6
AC1	2.03	96.5
YL 1/2	0.47	20.3
YL 1/2	1.01	4.4

A partially purified fraction from *Arbacia punctulata* blastulae was prepared and assayed for ribonucleotide reductase activity as described in Materials and Methods. 100% activity represents 0.91 nmol CDP converted to dCDP per milligram protein. A parallel extract of unfertilized eggs had 7% of this activity in this assay, though it was below the level of detection in others.

bition of activity. One was another anti-tubulin antibody, YOL 1/34 (29), which has a different specificity from YL 1/2 (57) and does not cross-react with p41 (reference 52 and our unpublished results). The other antibody tested was the monoclonal antibody AC1 described by Engström (17), which reacts with the large (M1) subunit of bovine ribonucleotide reductase. This antibody had no effect on the activity of the sea urchin enzyme. Passing sea urchin blastula extracts over the YL 1/2 affinity column likewise removed enzymic activity from the flow-through, whereas this was untrue of control columns (Dutia, B., and S. J. Bray, unpublished results). However, we have not yet tried to recover enzymic activity from this column, though this has been successful in the case of the mammalian enzyme (52) and would provide complete proof of identity.

Taking these results together with the earlier evidence that ribonucleotide reductase is specified by maternal mRNA (12, 37), we feel very secure in identifying the clam and urchin p41 as the small subunit of ribonucleotide reductase.

DISCUSSION

It is not by itself surprising that the mRNA for ribonucleotide reductase should be among the class of stored maternal mRNA. Earlier work had clearly shown that either ribonucleotide reductase itself, or an activator of the enzyme, must be encoded by maternal mRNA (12, 37). However, two things were unexpected: first, that the mRNA should be so abundant, and second, that only one such abundant protein was made after fertilization, given that ribonucleotide reductase is usually composed of two subunits, both of which are required for enzyme activity. If both subunits of the enzyme were newly made after fertilization, the large one (usually ~80,000 mol wt) would constitute by far the most strongly labeled band in early embryos, assuming that it had a reasonably normal content of methionine. We have also used leucine, phenylalanine, and tyrosine to label early embryos, and none of these amino acids shows up a new strongly labeled band of the expected size (reference 44 and our unpublished data). Thus it must be either that both clam and urchin ribonucleotide reductase lack a large subunit, or that the large subunit, unlike the small one, is stockpiled in the unfertilized egg. The latter seems to us the most likely explanation, for it is somewhat reminiscent of the regulation of ribonucleotide reductase activity in mammalian cells, in which the large subunit is present during all phases of the cell cycle in growing cells, whereas the small subunit and enzyme activity are present at high levels during S-phase, and at lower levels in G0 and G1 (19). However, there is clearly a need to characterize the subunit composition of ribonucleotide reductase from early embryonic stages of marine invertebrates in order to clarify this point.

In their pioneering studies of sea urchins, Noronha et al. (37) noticed that ribonucleotide reductase activity tended to decline after protein synthesis had been inhibited (though we have not detected significant proteolysis of p41 in analogous experiments in both clams and sea urchins, so instability may be due to inactivation rather than destruction). This provides perhaps the most plausible reason for stockpiling p41 mRNA rather than p41 itself: the protein would not be sufficiently stable to last during oogenesis, during which time it is not required in any case.

Quantitative Considerations

It is notoriously difficult to estimate absolute amounts of protein synthesis from incorporation data because of the uncertain size of the precursor pool. However, it would be interesting and helpful to know how much ribonucleotide reductase was synthesized, because the amount would serve as a standard for comparison with all the other polypeptides specified by maternal mRNA. We can make a rough estimate from the following considerations. The requirement for DNA precursors rises exponentially during the first few hours of embryogenesis; at the end of cleavage, the concentration of DNA is ~2 mg/ml, requiring ~3 mM dNTP precursors to be produced during S-phase. The total protein synthesis at this time is ~2 mg/ml per h (11), and we estimate that p41 initially constitutes ~5% of this total, falling as histone synthesis picks up (50). Thus the rate of ribonucleotide reductase synthesis is probably ~100 μg/ml per h for about the first 12 h of development. A more accurate estimate could be made if the turnover number of the clam or sea urchin enzyme were known.

Why Does p41 Bind to an Anti-Tubulin Antibody?

After the fortuitous discovery that p41 bound to the anti-tubulin antibody YL 1/2 we first directed our attention to this protein, in the mistaken idea that it might signify a role in spindle assembly. Thanks to the work of Wehland and colleagues, it is now clear why this binding occurs. The antibody recognizes the carboxy-terminal sequence of α-tubulin, -glu-gly-glu-glu-tyr. Removal of the carboxy-terminal tyrosine abolishes binding. However, replacement of the tyrosine by phenylalanine gives even tighter binding of tubulin to the antibody, and using model peptides to explore the specificity, Wehland et al. (56) found that aspartic acid can replace the penultimate glutamate residue; thus *E. coli* rec A protein, with a carboxy-terminal sequence of -thr-asn-glu-asp-phe competed very effectively with α-tubulin for binding to the antibody. Thus it is not surprising that p41, with the terminal sequence -thr-leu-asp-ala-asp-phe, is tightly bound. By contrast, HSV-2, EBV, and *E. coli* ribonucleotide reductase small subunits all lack a carboxy-terminal aromatic residue and do not bind to YL 1/2 (our unpublished observations, and Thelander, L. and B.-M. Sjöberg, personal communication). On the other hand, calf thymus M2 subunit binds well, and Thelander et al. have used the YL 1/2 affinity resin to purify this subunit to homogeneity (52).

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REFERENCES

1. Alexandraki, D., and J. V. Ruderman. 1984. Multiple, polymorphic α- and β-tubulin mRNAs are present in sea urchin eggs. *Proc. Natl. Acad. Sci. USA.* 82:134–138.
2. Alexandraki, D., and J. V. Ruderman. 1984. Tubulin gene expression in sea urchin embryogenesis. *Dev. Biol.* In press.
3. Anderson, C. W., P. R. Baum, and R. F. Gesteland. 1973. Processing of adenovirus 2-induced proteins. *J. Virol.* 12:241–252.
4. Bacchetti, S., M. J. Evelegh, B. Muirhead, C. S. Sartori, and D. Huszar. 1984. Immunological characterization of Herpes Simplex virus type 1 and 2 polypeptides involved in viral ribonucleotide reductase activity. *J. Virol.* 49:591–593.
5. Biggin, M. D., T. J. Gibson, and G. F. Hong. 1983. Buffer gradient gels and [^{35}S] label as an aid to rapid DNA sequence determination. *Proc. Natl. Acad. Sci. USA.* 80:3963–3965.
6. Brachet, J. 1967. Effects of hydroxyurea on development and regeneration. *Nature (Lond.).* 214:1132–1133.
7. Brandhorst, B. 1984. The informational content of the echinoderm egg. *In* Developmental Biology: A Comprehensive Synthesis. I. Oogenesis. L. Browder, editor. Plenum Publishing Corp., New York.
8. Bray, S. J., and T. Hunt. 1983. Developmental studies of a major maternal mRNA in *Arbacia punctulata. Biol. Bull.* 165:499–500.
9. Carlson, J., J. A. Fuchs, and J. Messing. 1984. The primary structure of the Escherichia coli ribonucleotide diphosphate reductase operon. *Proc. Natl. Acad. Sci. USA.* 81:4294–4297.
10. Cohen, G. H. 1972. Ribonucleotide reductase activity of synchronized KB cells infected with herpes simplex virus. *J. Virol.* 9:408–418.
11. Davidson, E. H. 1976. Gene Activity in Early Development. Academic Press, Inc., New York. 87–91.
12. De Petrocellis, B., and M. Rossi. 1976. Enzymes of DNA biosynthesis in developing sea urchins. *Dev. Biol.* 48:250–257.
13. Doolittle, R. F. 1981. Similar amino acid sequences: chance or common ancestry? *Science (Wash. DC).* 214:149–159.
14. Draper, K. G., R. J. Fink, and E. K. Wagner. 1982. Detailed characterization of an apparently unspliced Herpes Simplex virus type I gene mapping in the interior of another. *J. Virol.* 43:1123–1128.
15. Dutia, B. M. 1983. Ribonucleotide reductase induced by Herpes Simplex virus has a virus-specified constituent. *J. Gen. Virol.* 64:513–521.
16. Efstradiadis, A., F. C. Kafatos, A. M. Maxam, and T. Maniatis. 1976. Enzymatic in vitro synthesis of globin genes. *Cell.* 7:279–288.
17. Engström, Y. 1982. Monoclonal antibodies against mammalian ribonucleotide reductase. *Acta Chem. Scand. Ser. B Org. Chem. Biochem.* 36:343–344.
18. Engström, Y., S. Eriksson, L. Thelander, and M. Åkerman. 1979. Ribonucleotide reductase from calf thymus. Purification and properties. *Biochemistry.* 18:2941–2948.
19. Eriksson, S., and D. W. Martin, Jr. 1981. Ribonucleotide reductase in cultured mouse lymphoma cells. *J. Biol. Chem.* 256:9436–9440.
20. Evans, T., E. Rosenthal, J. Youngblom, D. Distel, and T. Hunt. 1983. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell.* 33:389–396.
21. Fansler, B., and L. A. Loeb. 1969. Sea urchin nuclear DNA polymerase. II. Changing localization during early development. *Exp. Cell. Res.* 57:305–310.
22. Galloway, D. A., L. C. Goldstein, and J. B. Lewis. 1982. Identification of protein encoded by a fragment of Herpes Simplex virus type 2 DNA that has transforming activity. *J. Virol.* 42:530–537.
23. Gibson, T., P. Stockwell, M. Ginsberg, and B. Barrell. 1984. Homology between two EBV early genes and HSV ribonucleotide reductase and 38K genes. *Nucleic Acids Res.* 12:5087–5099.
24. Holmgren, A. 1981. Regulation of ribonucleotide reductase. *Curr. Topics Cell. Reg.* 19:47–76.
25. Hultin, T. 1961. The effect of puromycin on protein metabolism and cell division in fertilized sea urchin eggs. *Experientia.* 17:410–411.
26. Huszar, D., and S. Bacchetti. 1981. Partial purification and characterization of the ribonucleotide reductase induced by Herpes simplex virus infection of mammalian cells. *J. Virol.* 37:580–588.
27. Huszar, D., and S. Bacchetti. 1983. Is ribonucleotide reductase the transforming function of Herpes Simplex virus 2? *Nature (Lond.).* 302:76–79.
28. Huszar, D., S. Beharry, and S. Bacchetti. 1983. Herpes Simplex virus-induced ribonucleotide reductase: development of antibodies specific for the enzyme. *J. Gen. Virol.* 64:1327–1335.
29. Kilmartin, J. V., B. Wright, and C. Milstein. 1982. Rat monoclonal antitubulin antibodies derived by using a new nonsecreting rat cell line. *J. Cell. Biol.* 93:576–582.
30. Kozak, M. 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes and organelles. *Microbiol. Rev.* 47:1–45.
31. Kuhn, O., and F. Wilt. 1981. Double labeling of chromatin proteins, in vivo and in vitro, and their two dimensional electrophoretic resolution. *Dev. Biol.* 85:416–424.
32. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
33. Mabuchi, I., and J. A. Spudich. 1980. Purification and properties of soluble actin from sea urchin eggs. *J. Biochem. (Tokyo).* 87:785–802.
34. Mathews, C. K. 1975. Giant pools of DNA precursors in sea urchin eggs. *Exp. Cell Res.* 92:47–56.
35. McLaughlan, J., and J. B. Clements. 1983. DNA sequence homology between two colinear loci on the HSV genome which have different transforming abilities. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:1953–1961.
36. Messing, J., and J. Veira. 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. *Gene.* 19:269–276.
37. Noronha, J. M., G. H. Sheys, and J. M. Buchanan. 1972. Induction of a reductive pathway for deoxyribonucleotide synthesis during early embryogenesis of the sea urchin. *Proc. Natl. Acad. Sci. USA.* 69:2006–2010.
38. Poccia, D., J. Salik, and G. Krystal. 1981. Transitions in histone variants of the male pronucleus following fertilization and evidence for a maternal store of cleavage-stage histones in the sea urchin egg. *Dev. Biol.* 85:416–424.
39. Ponstingl, H., E. Krauhs, M. Little, and T. Kempf. 1981. Complete amino acid sequence of α-tubulin from porcine brain. *Proc. Natl. Acad. Sci. USA.* 78:2757–2761.
40. Racine, F. M., and P. W. Morris. 1978. DNA polymerase α and β in the California urchin. *Nucleic Acids Res.* 5:3945–3957.
41. Raff, R. A. 1975. Regulation of microtubule synthesis and utilization during early embryonic development of the sea urchin. *Am. Zool.* 15:661–678.
42. Raff, R. A., and R. Showman. 1983. Maternal messenger RNA. *In* The Biology of Fertilization. C. B. Metz and A. Monroy, editors. Academic Press, Inc., New York.
43. Ricciardi, R., J. S. Miller, and B. E. Roberts. 1979. Purification and mapping of specific mRNAs by hybridization-selection and cell-free translation. *Proc. Natl. Acad. Sci. USA.* 76:4927–4931.
44. Rosenthal, E., T. Hunt, and J. V. Ruderman. 1980. Selective translation of mRNA controls the pattern of protein synthesis during early development of the surf clam, *Spisula solidissima. Cell.* 20:487–496.
45. Rosenthal, E. T., T. R. Tansey, and J. V. Ruderman. 1983. Sequence-specific adenylations and deadenylations accompany changes in the translation of maternal mRNA after fertilization of Spisula oocytes. *J. Mol. Biol.* 166:309–327.
46. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain termination inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.
47. Schiff, P. V., J. Fant, and S. B. Horvitz. 1979. Promotion of microtubule assembly in vitro by taxol. *Nature (Lond.).* 277:665–667.
48. Sjöberg, B.-M., H. Eklund, J. A. Fuchs, J. Carlson, J. Messing, N. Standart, S. Bray, T. Hunt, and J. V. Ruderman. 1985. Identification of the free radical tyrosine residue in ribonucleotide reductase. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* In press.
49. Staden, R. 1982. An interactive graphics program for comparing and aligning nucleic acid and amino acid sequences. *Nucleic Acids Res.* 10:2951–2961.
50. Tansey, T. R., and J. V. Ruderman. 1983. Differential mRNA accumulation and translation during Spisula development. *Dev. Biol.* 99:338–351.
51. Thelander, L., and P. Reichard. 1979. Reduction of ribonucleotides. *Annu. Rev. Biochem.* 48:188–158.
52. Thelander, M., A. Gräslund, and L. Thelander. 1985. Subunit M2 of mammalian ribonucleotide reductase. Characterization of a homogeneous protein isolated from M2-overproducing mouse cells. *Proc. Natl. Acad. Sci. USA.* In press.
53. Timourian, H., and J. Uno. 1967. Protein synthesis requirements for the first two cleavages in sea urchin eggs. *Exp. Cell Res.* 48:173–176.
54. Vallee, R. 1982. A taxol-dependent procedure for the isolation of microtubules and microtubule-associated proteins (MAPS). *J. Cell Biol.* 92:435–442.
55. Wagenaar, E. B. 1983. The timing of synthesis of proteins required for mitosis in the cell cycle of the sea urchin embryo. *Exp. Cell Res.* 144:393–403.
56. Wehland, J., H. C. Schroeder, and K. Weber. 1984. Amino acid sequence requirements in the epitope recognized by the α-tubulin-specific rat monoclonal antibody YL 1/2. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1295–1300.
57. Wehland, J., M. C. Willingham, and I. V. Sandoval. 1983. A rat monoclonal antibody reacting specifically with the tyrosylated form of α-tubulin. I. Biochemical characterization, effects on microtubule polymerization in vitro, and microtubule polymerization and organization in vivo. *J. Cell Biol.* 97:1467–1475.
58. Wilt, F. H., H. Sakai, and D. Mazia. 1967. Old and new protein in the formation of the mitotic apparatus in cleaving sea urchin eggs. *J. Mol. Biol.* 27:1–7.
59. Young, C. W., I. J. Hendler, and D. A. Karnofsky. 1969. Synthesis of protein for DNA replication and cleavage events in the sand dollar embryo. *Exp. Cell Res.* 58:15–26.