**The structure of ribonucleotide reductase complex**

The three-dimensional structure of the large subunit of Class I RNR in *E. coli* has been determined, and it serves as the model of RNR in *E. coli* and eukaryotes (Figure 5)(Nordlund et al. 1990; Nordlund and Eklund 1993; Uhlin and Eklund 1994). This enzyme consists of two identical large subunits, or a homodimer of $\alpha_2$ (171 kD, 2 x 761 residues) and two identical small subunits, or a homodimer of $\beta_2$ (87 kD, 2 x 375 residues). One large and one small subunit form an active site between them, so the enzyme complex has two active sites. At each active site, the large subunit contributes sulfhydryl groups from cysteines; the small subunit provides a tyrosyl free radical ($Tyr^{\cdot}$).

In addition to its sulfhydryl pair, each large subunit contains two allosteric sites: an overall activity site and a substrate specificity site (Thelander and Reichard 1979). The overall activity site controls the general supply of dNTPs for DNA synthesis. Binding to the overall activity site, dATP (a product of RNR) inhibits the enzyme activity by feedback, whereas ATP enhances the enzyme activity. The substrate specificity site regulates the balance between the four ribonucleoside diphosphates. Binding to the substrate specificity site, a nucleotide enhances the synthesis of another nucleotide: dGTP enhances dADP synthesis; dTTP enhances dGTP synthesis; dATP and ATP enhance dCTP and dUTP synthesis.

The three-dimensional structure of the large subunit of RNR in *E. coli* has been determined (Uhlin and Eklund 1994)

(Figure 6). Two redox-active cysteines, Cys 225 and Cys 462, are directly involved in the substrate reduction. Although they are far away from each other in amino acid sequence, they are adjacent at the active site of the three-dimensional structure. Another cysteine at the active site, Cys 439, may form a cysteine free radical. This free radical may draw away the H atom ($H^{\cdot}$) at the 3' C of the ribose moiety to start the reaction.

The large subunits of RNR have conserved amino acid residues, which are the same in other organisms (Uhlin and Eklund 1994). A few extremely conserved residues are illustrated with the residues of the large subunit of RNR in *E.coli*. They are the cysteines for reducing the ribose of the substrate (Cys 225, 439, 462), the tyrosines for the transferring the free radical from the small subunit (Tyr 730,731), and the amino acid residues for binding and transforming the ribose moiety of the substrate (Ser 224, Asn 437, Glu 441). These conserved amino acid residues are features that help identify the large subunit of RNR.

Besides its tyrosyl free radical, each small subunit contains a cofactor or a prosthetic group. The cofactor, two iron ions connected by an oxygen atom, affects a nearby tyrosine to form the tyrosyl free radical.

The three-dimensional structure of the small subunit of RNR in *E. coli* has been determined (Nordlund et al. 1990; Nordlund and Eklund 1993) (Figure 7). Its basic recurrent motifs are eight long helices. The cofactor is buried within the subunit. Above this cofactor and near the

surface (10 Å from the surface) is the tyrosyl free radical (Tyr 122), which is essential for the catalytic activity.

The small subunit of RNR has 17 extremely conserved amino acid residues (Nordlund and Eklund 1993). All these 17 amino acid residues are identical in at least 10 organisms examined. These 17 amino acid residues are illustrated with the residues of the small subunit of RNR in *E.coli*. They are the iron ligands residues and its environment (Trp 48, Glu 115, His 118, Glu 204, Asp 237, Glu 238; His 241), the tyrosyl radical and its environment (Tyr 122; Phe 208, Ser 211, Phe 212; Ile 234), the residues for the binding of the large subunit (Asp 59, Arg 236, Glu 350, Tyr 356), and the residue for a helix turn (Pro 331). These conserved amino acid residues are features that help identify the small subunit of RNR.

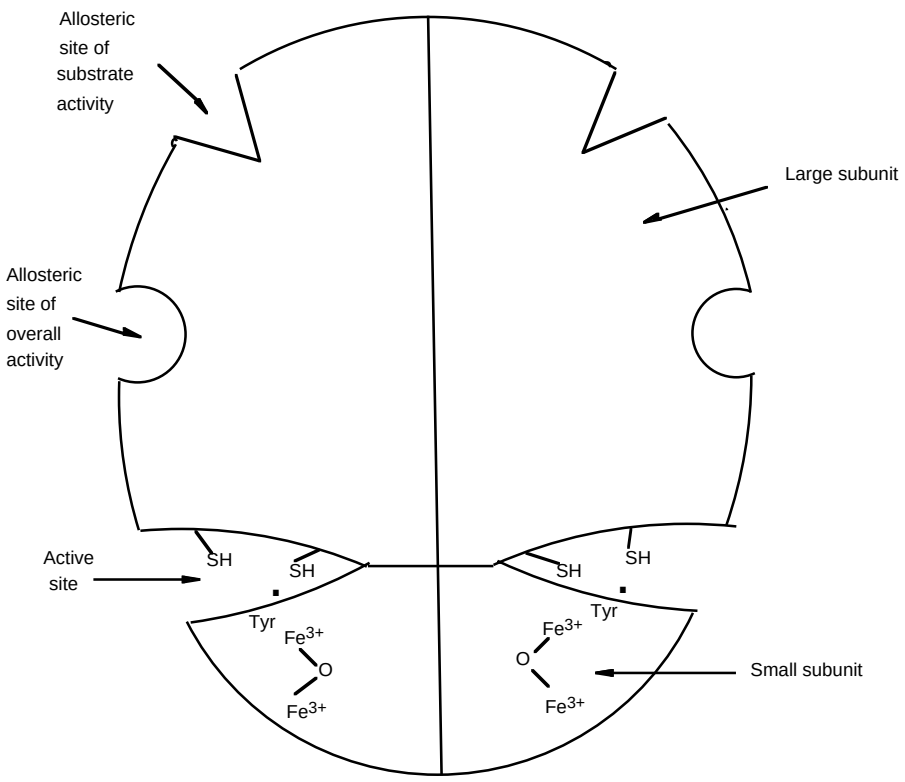


**Figure 5. The conceptual structure of ribonucleotide reductases in *E. coli* and eukaryotes**

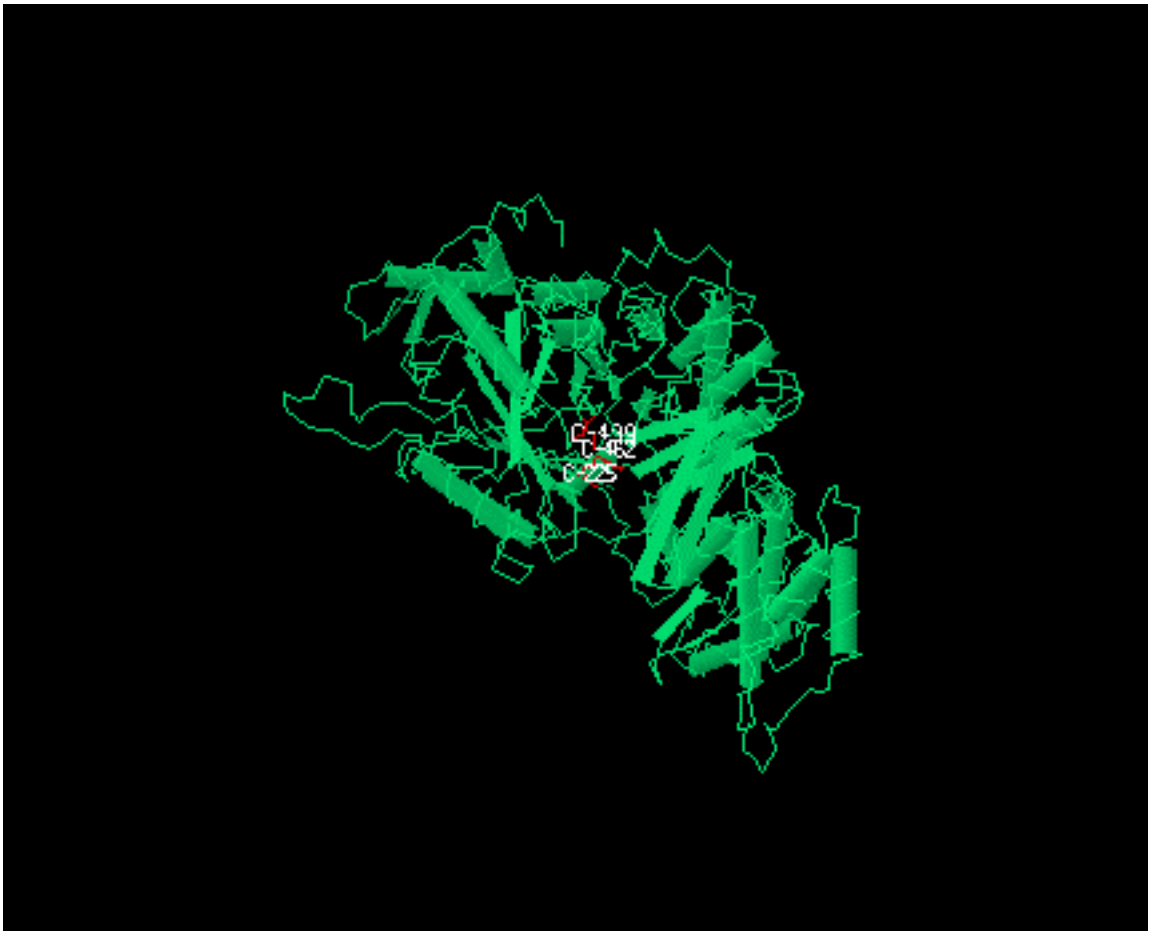

**Figure 6. The three-dimensional structure of the large subunit of ribonucleotide reductase in *E. coli*.**
A single large subunit is shown. Three redox-active cysteines, C-225, C-462 and C-439, are shown in red. Although they are far away from each other in amino acid sequence, they are adjacent at the active site of the three-dimensional structure. The structure originates from 1RLR in the Molecular Modeling Database at the National Center for Biotechnology Information (NCBI); then it was manipulated with Cn3D, a 3D molecular structure viewing and analysis program from NCBI.

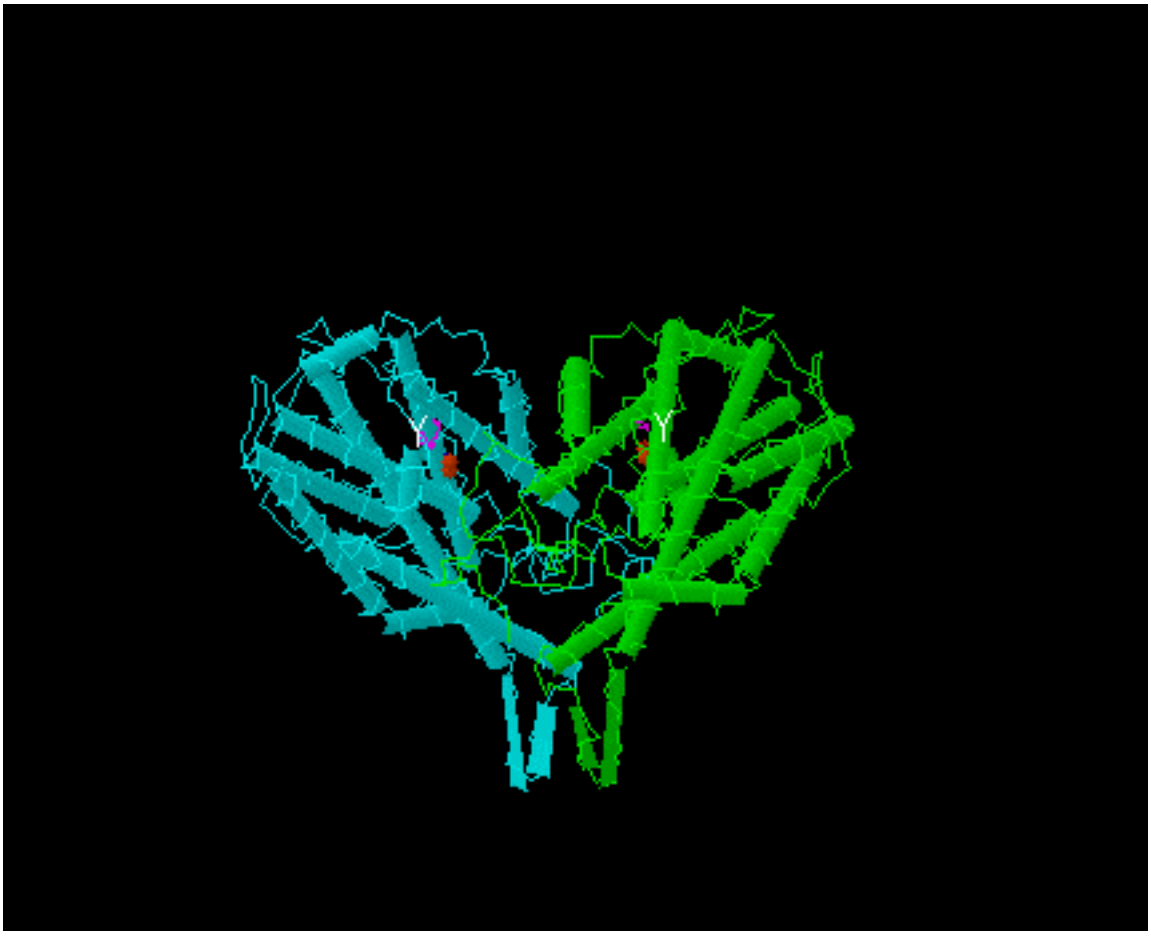


**Figure 7. The three-dimensional structure of the small subunit of ribonucleotide reductase in *E. coli*.**
Two small subunits, or a homodimer, are shown in blue and green respectively. The cofactor is buried within the subunit (red). Above the cofactor and near the surface is the tyrosine 122 residue (purple, marked Y), which is essential for the catalytic activity. The structure originates from 1AV8_B in the Molecular Modeling Database at the National Center for Biotechnology Information (NCBI); then it was manipulated with Cn3D, a 3D molecular structure viewing and analysis program from NCBI.

**Clones and sequences of ribonucleotide reductases in eukaryotes**

Subunits of RNR have been cloned and sequenced from many eukaryotic organisms (Table 2). However, little was known about RNR in plants a few years ago (Chaboute et al. 1998). When this study began in 1994, RNR clones and sequences in plants had not been reported. In 1995, other investigators published the cDNA clone and sequence of the small subunit of RNR in *Arabidopsis* (Philipps et al. 1995). By 1997, I reported to my advisory committee the cDNA clones and sequences of the complete large and small subunit of RNR in soybean. The sequence of the small subunit of RNR in soybean has been published (Xiong and Cowles 1999). Other investigators published the cDNA clones and sequences of the large and small subunits of RNR in tobacco (Chaboute et al. 1998).

**Table 2. Clones of ribonucleotide reductase in eukaryotes**

| Organism | Subunit | Reference |
|---|---|---|
| human | large,small | (Parker et al. 1991) (Pavloff et al. 1992) |
| mouse | large,small | (Caras et al. 1985) (Thelander and Berg 1986) |
| hamster | small | (Chaudhuri et al. 1992) |
| zebrafish | large,small | (Mathews et al. 1996) |
| fruit fly | large,small | (Duronio and O'Farrell 1994) |
| nematode | large | (Wilson et al. 1994) |
| clam | small | (Standart et al. 1985) |
| *Urechis* | small | (Rosenthal 1993) |
| baker's yeast | large<br>small | (Elledge and Davis 1990) (Yagle and McEntee 1990) (Elledge and Davis 1987)<br>(Hurd et al. 1987)<br>(Wang et al. 1997) |
| fission yeast | large,small | (Fernandez Sarabia et al. 1993) |
| *Leishmania* | small | (Lye et al. 1997) |
| Plasmodium | large,small | (Chakrabarti et al. 1993)<br>(Rubin et al. 1993) |
| *Dictyostelium* | small | (Tsang et al. 1996) |

| | | |
|---|---|---|
| *Trypanosoma* | large,small | (Dormeyer et al. 1997)<br>(Hofer et al. 1997) |
| *Arabidopsis* | large<br>small | (Lin et al. 1999)<br>(Philipps et al. 1995) |
| tobacco | large,small | (Chaboute et al. 1998) |

**The gene or gene family encoding the subunits of ribonucleotide reductase**

Although only one large and one small subunit of RNR have been found in most eukaryotes, there are reports that a gene family encodes different large or small subunits of RNR in a few eukaryotes. In baker's yeast, two genes encode two RNR large subunits with about 80% amino acid identity between them (Elledge and Davis 1990). One gene is essential for viability and is regulated with cell cycle. The other gene is unessential for viability and is strongly expressed following DNA damage. Likewise in baker's yeast, two genes encode two RNR small subunits with about 52% amino acid identity (Elledge and Davis 1987; Hurd et al. 1987; Wang et al. 1997). One small subunit has catalytic activity; the other does not have catalytic activity, but is required for the enzyme complex. It is not clear how these different large and small subunits are selected to form the enzyme complex in yeast.

Recent findings from plants show that a small gene family encodes different large subunits of RNR in tobacco (Chaboute et al. 1998), whereas a single gene encodes the only small subunit of RNR in tobacco (Chaboute et al. 1998) and in *Arabidopsis* (Philipps et al. 1995). Whether a gene family or a single gene encodes the large and small subunit of RNR in soybean is part of this study.

**Gene expression of ribonucleotide reductase in *E. coli* and in eukaryotes**

In *E. coli* cells, RNR gene expression is cell-cycle dependent (Sun and Fuchs 1992). An operon, *nrd*, encodes both the large and small subunit of RNR, and it is transcribed into a polycistronic RNR mRNA. In each generation of synchronized *E. coli* cells, the RNR mRNA increases when DNA synthesis starts; it decreases back to basal levels soon after DNA synthesis starts. This cell-cycle regulation of *nrd* expression is controlled by five upstream cis-acting elements (Jacobson and Fuchs 1998).

In mouse cells, RNR gene expression is cell-cycle dependent (Bjorklund et al. 1990) and inducible by UV-light treatments that damage DNA (Filatov et al. 1996). Both the mRNA encoding the large subunit and that encoding the small subunit are very low or undetectable during $G_0/G_1$ phase. They are high during S phase and low again during $G_2$+M phase. By UV-light treatments, the promoter of the gene encoding the large subunit is induced up to 3-fold; the promoter of the gene encoding the small subunit is induced up to 10-fold. The transcription of the promoter of both genes is controlled by multiple protein-DNA interactions (Jordan and Reichard 1998). The small subunit of RNR is phosphorylated by cyclin-dependent kinases P34cdc2 and CDK2 (Chan et al. 1999). Over expression of the small subunit enhances malignant potential of mouse cells (Fan et al. 1996), but the large subunit suppress the malignant potential (Fan et al. 1997). Despite some phenomena, the

relationship between RNR and cell cycle regulation and checkpoints is still unclear.

In baker's yeast, RNR gene expression is cell-cycle dependent, and it is inducible by treatments that damage DNA or block DNA replication (Elledge et al. 1992). Gene *RNR1* and *RNR3* encode two different large subunits of RNR. Gene *RNR2* and *RNR4* encode two different small subunits of RNR. Through the cell cycle the mRNA levels of *RNR1* fluctuates 10-fold; and the mRNA levels of *RNR2* fluctuates 2-fold. By treatments that damage DNA or block DNA replication, *RNR1* is induced 5-fold; *RNR2* is induced 25-fold; and *RNR3* is induced 500-fold.

Not much is known about RNR gene expression in plants. RNR gene expression in tobacco is cell-cycle dependent (Chaboute et al. 1998). In synchronized tobacco cells, the mRNA levels of both the large and small subunit are high in the S phase and low in the $G_2$, M and $G_1$ phases.

In intact plant tissue, there are no reports of RNR gene expression affected by environmental conditions. It is well-known that if young soybean seedlings were grown in the dark, the primordial shoots remain yellow and in bud. However, after transferring to light for a couple of hours, the primordial shoots begin to green, grow and develop. This phenomenon implies that light may turn on or increase some genes for cell division. Because RNR is a key enzyme for DNA synthesis and cell division, I want to know if RNR genes in soybean are expressed both in the dark-grown and light-grown seedlings, and if lighting turns on or

increases the mRNA levels. I estimated RNR mRNA levels in primordial shoots of young soybean seedlings by Northern hybridization.

**Multiple poly(A) sites in plant mRNA**

Almost all eukaryotic mRNA has a 3' poly(A) tail. In the nucleus, the pre-mRNA loses its 3'-end by endonucleolytic cleavage and then obtains a 3' poly(A) tail by polymerization of AMP (Minvielle-Sebastia and Keller 1999; Wahle and Ruegsegger 1999; Zhao et al. 1999). The poly(A) tail of mRNA functions in exporting the mRNA from the nucleus to the cytoplasm, initiating translation, and regulating mRNA stability.

Animal mRNAs normally have a single poly(A) site and three cis-acting elements locate the site. The first element is a highly conserved AAUAAA sequence, found 10-30 nucleotides upstream from the poly(A) site. The second element is a U-rich or GU-rich sequence, found usually within 30 nucleotides downstream from the poly(A) site. The third element is the cleavage site, or poly(A) site itself. A CA dinucleotide is frequently found just before the cleavage site. So the first adenine ribonucleotide of the poly(A) tail in an animal mRNA is likely gene-encoded (Zhao et al. 1999).

Based on a rather small number of functional studies, it is proposed that three cis-acting elements locate the poly(A) site in plants (Wu et al. 1995; Rothnie 1996; Li and Hunt 1997). The first element is a near-upstream element, found 10-40 nucleotides upstream from the poly(A) site. It is an AAUAAA-like or a rather unrelated sequence. The second element is a far-upstream element, found 13 to 100 nucleotides upstream from the near-upstream element; it is usually U-rich. The third element is the cleavage site, or poly(A) site itself. A dinucleotide Y(= C or T)A is frequently found at the poly(A) site. The cis-acting elements locating the plant poly(A) sites differ between mRNAs and are hard to discern by sequence inspection alone.

Many plant mRNAs have multiple poly(A) sites (Wu et al. 1995; Rothnie 1996; Li and Hunt 1997). For example, 14 poly(A) sites were found from 22 cDNA clones encoding a chloroplast RNA-binding protein in tobacco (Klahre et al. 1995). These multiple poly(A) sites were conformed by RNase A/T1 mapping of tobacco RNA. Some researchers consider multiple cis-acting elements as the cause for multiple poly(A) sites in plants (Li and Hunt 1997). To determine possible multiple poly(A) sites of RNR mRNAs in soybean, I sequenced multiple clones containing 3' ends of the cDNA encoding the large and small subunits of RNR in soybean.

# Materials and Methods

## Plant material

The soybean (*Glycine max*) used in this study was the Cultivar “Chesapeake”, which was kindly provided by Dr. Glenn Buss, Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. The soybean seeds were germinated in potting soil in an incubator at 20°C in the dark for four days.

All nucleic acid was isolated from the primordial shoots of young soybean seedlings. Genomic DNA was isolated after the seeds were germinated for four days and the seedlings emerged from the potting soil in the dark. Total RNA was isolated after the seeds were germinated for four days, and the seedlings emerged from the potting soil in the dark and then grew in the light (4000 food candle) for three hours. Poly(A)$^+$ RNA was isolated after the seeds were germinated for four days, and the seedlings emerged from the potting soil in the dark and then grew either in the dark or in the light (4000 foot candle) for three hours.

## Isolation of DNA and RNA

For genomic DNA isolation, the primordial shoots (2-3 mm long) were ground to a fine powder in liquid nitrogen. One gram tissue powder was solubilized in a guanidinium

thiocyanate solution (1 g tissue/3 ml DNAzol ES, Molecular Research Center, Cincinnati, OH), extracted with chloroform (3 ml) and centrifuged (4° C, 12,000 x *g*, 10 min). The supernatant was collected. DNA was precipitated with ethanol (0.75 volume of 100% ethanol) and collected by centrifugation (4° C, 5,000 x *g*, 4 min). The pellet was suspended in RNase A solution (400 µl, 10 µg/µl in 5 mM EDTA) and incubated at 37°C for 15 min. The incubated suspension was washed with a DNAzol ES-ethanol mixture (1:0.75 volume, 4 ml) and centrifuged at 5,000 x *g* for 4 min. The pellet was washed with ethanol (3 ml, 90% ethanol) and centrifuged at 5,000 x *g* for 4 min. The ethanol washing was repeated once. The DNA pellet was briefly air-dried (10 min), dissolved in NaOH (200 µl, 8 mM) and centrifuged at 12,000 x *g* for 4 min. The DNA solution was neutralized with HEPES (4.6 µl, 1 M free acid) to pH 7.2.

Total RNA was isolated as described by Verwoerd et al. (Verwoerd et al. 1989). Primordial shoots (2-3 mm long, 1 g) were ground to a fine powder in liquid nitrogen. The tissue powder was homogenized in hot exaction buffer [phenol – 0.1 M LiCl, 100 mM Tris.HCl pH = 8.0, 10 mM EDTA, 1% SDS (1:1), 80°C, 5 ml] by vortex (30 sec), extracted with chloroform-isoamylalcohol (24:1, 2.5 ml) and centrifuged (4° C, 10,000 x *g*, 10 min). The supernatant was collected and RNA was precipitated with LiCl (4 M, 1 volume, 4° C, for 15 min) and centrifuged (4° C, 10,000 x *g*, 30 min). The RNA was suspended in water (2.5 ml), precipitated with NaOAc (3 M, pH = 5.2, 0.1 volume) and ethanol (2 volumes), and centrifuged (4° C, 10,000 x *g*, 30 min). The RNA was washed

in ethanol (2 ml, 75% ethanol), centrifuged at 10,000 x *g* for 10 min, and dried.

Poly(A) RNA was isolated in a guanidinium thiocyanate solution and oligo(dT) cellulose (Poly(A)Pure, Ambion, Austin, Texas) according to the manufacturer's protocol. Primordial shoots (2-3 mm long, 1 g) were ground to a fine powder in liquid nitrogen. The tissue powder was homogenized in Lysis Solution (offered in the kit, 10 ml), diluted with Dilution Buffer (offered in the kit, 20 ml), and centrifuged (RT, 12,000 x *g*, 15 min). The supernatant was transferred to a fresh tube. Oligo dT cellulose (one vial T18 resin) was added to the supernatant. The mixture was incubated with continual rocking for 60 min and centrifuged (RT, 4,000 x *g*, 5 min). After the supernatant was removed, the oligo dT with bound mRNA was washed in a high salt solution (10 ml Binding Buffer, offered in the kit) and centrifuged (RT, 4,000 x *g*, 3 min). Then the oligo dT with bound mRNA was washed in a low salt solutions (10 ml Wash Buffer, offered in the kit) and centrifugation (RT, 4,000 x *g*, 3 min; discard supernatant; repeat 3 times). The oligo dT with bound mRNA was collected in a spin column. The column was washed with a low salt solutions (500 µl Wash Buffer, offered in the kit) and centrifuged (RT, 5,000 x *g*, 10 sec). The washing was repeated (3 or more times) until the $A_{260}$ of the flow-through was ≤ 0.05. The mRNA was eluted by Elution Buffer (offered in the kit, at 65°C, 200 µl) and centrifuged (RT, 4,000 x *g*, 30 sec).

**Cloning and sequencing of the cDNAs**

For cloning of RNR subunits in soybean, a PCR and clone strategy was used (Figure 8), which included PCR with highly degenerate primers and Rapid Amplification of CDNA Ends technique (RACE).

First, soybean cDNA was reverse transcribed (Figure 8) according to the manufacturer's protocol (Superscript, Gibco BRL, Rockville, MD). Total soybean (Glycine max) RNA (as template), oligo(dT) (as primer), and reverse transcriptase from murine leukemia virus were used for reverse transcription. Total soybean RNA (10 µg) and oligo(dT) (5 µM, 8 µl) were mixed (42 µl total), incubated at 70°C for 5 min, and chilled on ice water for 2 min. To the above mixture, PCR buffer (10x, 8 µl), $MgCl_2$ (25 mM, 8 µl), dNTP mix (10 mM, 4 µl), DTT (0.1 M, 8 µl), and RNasin (2 U/2 µl) were added. The new mixture was incubated at 50°C for 5 min. Reverse transcriptase (Superscript II RT, 200 U/1 µl) was added. The new mixture was incubated at 50°C for 60 min; and then at 94°C for 5 min. RNase H (2 U/1 µl) was added and the new mixture was incubated at 37°C for 20 min. The final mixture (80 µl total) containing first strand cDNA was stored at –20°C before it was used as template for PCR amplification.

Second, the first segment of the cDNA encoding a RNR subunit was amplified by PCR (Figure 8). Transcribed soybean cDNA was used as template for the PCR. After a single PCR product with an expected size was confirmed by agarose gel electrophoresis, it was cloned and sequenced.

The sequence was used to identify the clone encoding the expected part of the subunit. The sequence was also used to design primers, with which the 3' and 5' ends of the cDNA were amplified.

Highly degenerate primers were used in the PCR to amplify the first segment of the cDNAs (Table 3,4,5). Because little was known about RNR in plants, the primers were designed according to the conserved regions of known RNR in other eukaryotes: human, mouse, hamster, clam, nematode, bakers' yeast, fission yeast, and plasmodium. Expressed Sequence Tags of Arabidopsis were also used. In the conserved regions of the known RNR in these organisms, the amino acid sequences are relatively conserved, but the DNA sequences are still degenerate. For example, an upstream primer with a degeneracy of 16,384 fold might be needed to amplify the first segment of the cDNA encoding Large Subunit A (Table 4). A primer mixture with 16,384 different sequences could achieve this high degeneracy, but so many different primers would interact with each other, form primer dimers, and produce wrong PCR products. Matching A, C, G or T, deoxyinosine was used to maintain the high degeneracy but decrease the variety of the primer. Five nucleotides of deoxyinosine were used in this primer, and the variety was decreased one thousand times, from 16,384 to 16. Degenerate primers are shown in accordance with the International Union for Pure and Applied Chemistry (IUB) code (Table 3,4,5).

Third, the 3' and 5' ends of the cDNAs were amplified (Figure 8). Rapid Amplification of cDNA Ends, or RACE

techniques, were used according to the manufacturer's protocol (Marathon cDNA Amplification, 3'-AmpliFinder RACE, Clontech, Palo Alto, CA) (Tables 3,4,5). Two rounds of PCR were carried out during the RACE procedure. These 3' and 5' ends were cloned and sequenced. The sequences were used to identify the clones encoding the expected 3' or 5' ends and to design primers, with which the complete coding region of the cDNA was amplified.

Finally, the complete coding region of the cDNA was amplified by PCR with soybean cDNA (as template), the upstream-start-codon-primer and the downstream-stop-codon-primer (Figure 8)(Tables 3,4,5). The upstream-start-codon-primer targeted the region upstream from the start codon of the cDNA. It was designed according to the 5' ends of the cDNA. The downstream-stop-codon-primer targeted the region downstream from the stop codon of the cDNA. It was designed according to the 3' ends of the cDNA. Part of the cDNA encoding a third large subunit (Large Subunit C) was unintentionally amplified when the 3' end of the cDNA encoding the Large Subunit B was amplified (Table 5).

cDNAs were amplified with Taq DNA polymerase or a DNA polymerase mixture. When a short segment of cDNA (<1,000 bp) was amplified, Taq DNA polymerase (PCR Core Kit, Boehringer Mannheim, Indianapolis, IN) was used. When a long segment of cDNA (>1,000 bp) or a cDNA including complete coding region was amplified, a DNA polymerase mixture with proofreading activity (Expand Long Template PCR System, Boehringer Mannheim, Indianapolis, IN) was used to reduce the error rate of the amplification.

PCR-amplified cDNAs encoding the subunits of RNR were cloned with either one of two kits as suggested by the respective manufacturers. The cDNA encoding the small subunit was cloned into blunt-end vectors with T4 DNA ligase (Prime PCR Cloner kit, 5 Prime -> 3 Prime). The cDNAs encoding the large subunits of RNR were cloned into TA vectors with topoisomerase according to the manufacturer's protocol (TOPO TA Cloning kit, Invitrogen, Carlsbad, CA). The cloning mixture included PCR product (1 µl), vector (pCR TOPO Vector, 1 µl, 10 ng) and water (3 µl). The cloning mixture was incubated at RT for 5 min. ß-mercaptoethanol (0.5 M, 2 µl) was added into competent cells (One Shot cells, a vial). The cloning mixture (2 µl) was added into the competent cells (One Shot cells, a vial) and the cells were incubated on ice for 30 min. The cells was heat-shocked (42°C, 30 sec) and then incubated on ice for 2 min. SOC medium (RT, 250 µl) was added into the cells. After incubation (37°C, agitated at 225 cycles/min, 30 min), the cells (50 µl each plate) was spread on medium plates (LB, 100 mg/ml ampicillin).

cDNA clones were sequenced manually or by a commercial DNA sequencing service. cDNA clones were sequenced manually with dideoxynucleotide chain-termination method (Sanger et al. 1977) and DNA polymerase (Sequenase™ Version 2.0 T7, USB, Cleveland, OH). cDNA clones were sequenced by commercial DNA sequencing service with ABI Prism 377XL automated DNA sequencer (DNA Sequencing Facility, University of Chicago Cancer Research Center, Chicago, IL).

The accuracy in the sequences was assured and the sequence data were analyzed. Accuracy in the sequence of the coding region was assured by sequencing five different clones encoding the same subunit of RNR. More than 99% of the bases had identical base reading in all five clones; the other less than 1% of the bases had the same base reading at four out of the five clones. Sequence data were analyzed with Lasergene Biocomputing Software (DNAStar, Madison, WI) and Basic Local Alignment Search Tool (BLAST) programs (Altschul et al. 1990).

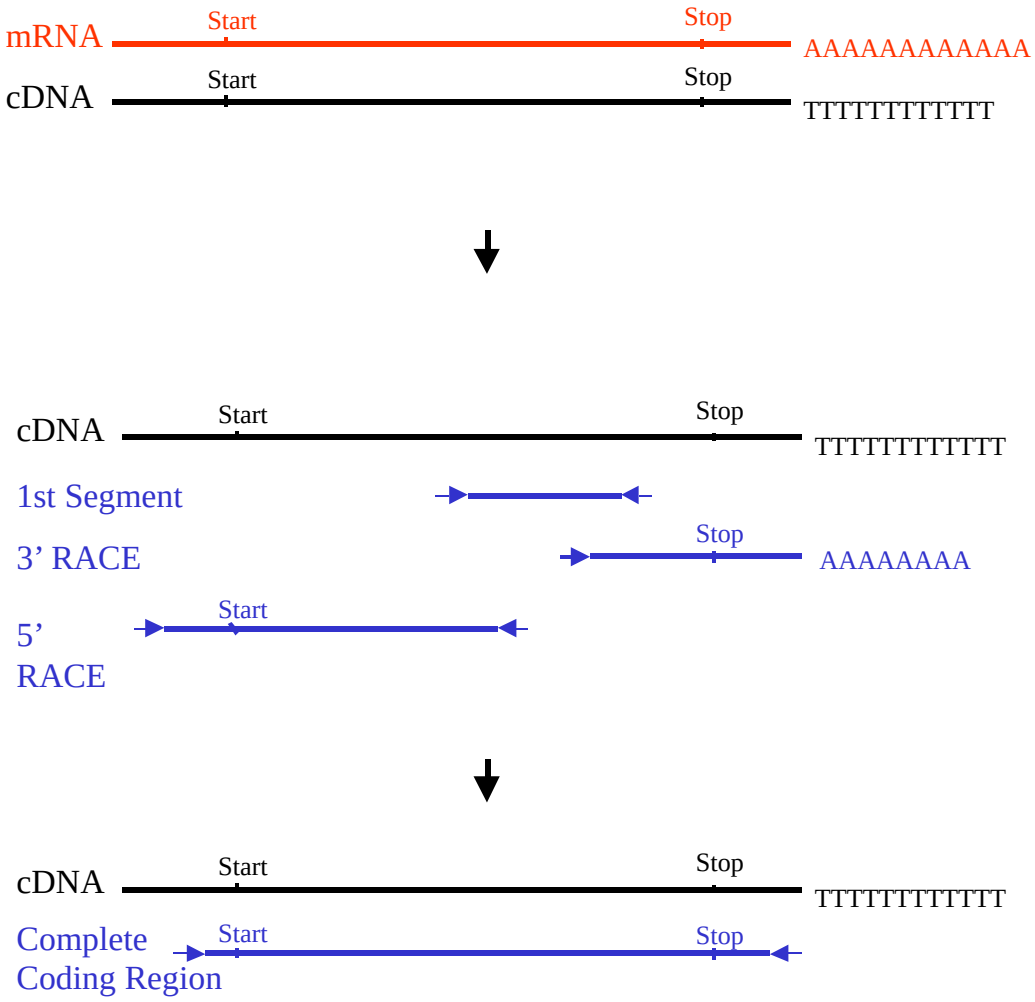


**Figure 8. A PCR and clone strategy for cloning a RNR subunit in soybean**