cDNA Cloning and Gene Characterization of Large and Small Subunits of Ribonucleotide Reductase in Soybean

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Abstract

Ribonucleotide reductase (RNR) reduces four ribonucleoside diphosphates to corresponding deoxyribonucleoside diphosphates, which are transformed into deoxyribonucleoside triphosphates, substrates for DNA polymerase. By controlling the supply and balance of deoxyribonucleoside diphosphates, RNR regulates DNA synthesis. RNR in *E. coli* and in animals consists of two identical large and two identical small subunits. Until recently, little was known about RNR in plants. For cloning RNR cDNA in plants, soybean (*Glycine max*) cDNAs were amplified with highly degenerate primers and the Rapid Amplification of cDNA Ends techniques. The cDNAs encoding two complete large subunits, one partial large subunit and one complete small subunit of RNR in soybean were cloned and sequenced. The RNR large subunits in soybean contain a motif with 20 amino acids, which appears to be specific for the RNR large subunits in plants. Southern hybridization results imply that a gene family encodes at least three different large subunits of RNR in soybean, and that a single gene encodes the small subunit. The presence of

three different large subunits of RNR in soybean suggests that RNR complex in some plants may have a non-homodimer structure; alternatively, some plants may have different RNR isozymes. Northern hybridization results show that RNR large and small subunit genes in soybean are expressed both in dark-grown and light-grown seedlings, and that light does not increase RNR mRNA levels. Multiple poly(A) sites and different lengths of the 3’ untranslated regions were found in cDNAs encoding some subunits of RNR in soybean. The same cis-acting elements may imprecisely locate some multiple poly(A) sites in plants.

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Introduction

Ribonucleotide reductase - an essential enzyme for DNA synthesis

Ribonucleotide reductase (RNR) reduces ribonucleoside diphosphates to deoxyribonucleoside diphosphates, which are transformed into deoxyribonucleoside triphosphates, substrates for DNA polymerase (Figure 1)(Reichard 1993). This single enzyme reduces four ribonucleoside diphosphates to corresponding deoxyribonucleoside diphosphates (Figure 2) (Reichard 1988). By controlling the supply and balance of deoxyribonucleoside diphosphates, RNR regulates DNA synthesis.

The catalytic reaction of ribonucleotide reductase

RNR replaces the hydroxyl group at the 2' position of the ribose with a hydrogen atom (Figure 3) (Stubbe 1990). RNR donates electrons from its sulfhydryl group and converts itself into an oxidized form. To return to its reduced form, the oxidized RNR accepts electrons from thioredoxin or glutaredoxin. Then the oxidized thioredoxin accepts electrons from thioredoxin reductase, whereas the oxidized glutaredoxin accepts electrons from glutaredoxin reductase. Both oxidized thioredoxin reductase and oxidized glutaredoxin reductase accept electrons from NADPH.

The proposed reaction mechanism of RNR can be divided into four steps (Figure 4) (Stubbe 1990). In the first step, the H atom ($H^{\cdot}$) at the 3' C of the ribose moiety is drawn away by a RNR free radical. This RNR free radical may be a cysteine free radical on the large subunit and is promoted by a tyrosyl free radical on the small subunit. In the second step, the OH^{-} group at 2' C of the ribose moiety is drawn away by a H^{+} released from the sulfhydryl group on RNR. This step is possible because the unpaired electron on the 3' $C^{\cdot}$ weakens the bond between the 2' C and its OH^{-} group. The 3' $C^{\cdot}$ is the result of the loss of the H atom at the 3' C. After the second step, the ribose moiety forms a radical cation. In the third step, the radical cation is reduced when an H^{-} is donated to the 2' carbon of the ribose moiety from the sulfhydryl group on RNR. In the fourth step, the original H atom is returned to the 3' carbon of the ribose moiety from the RNR free radical.

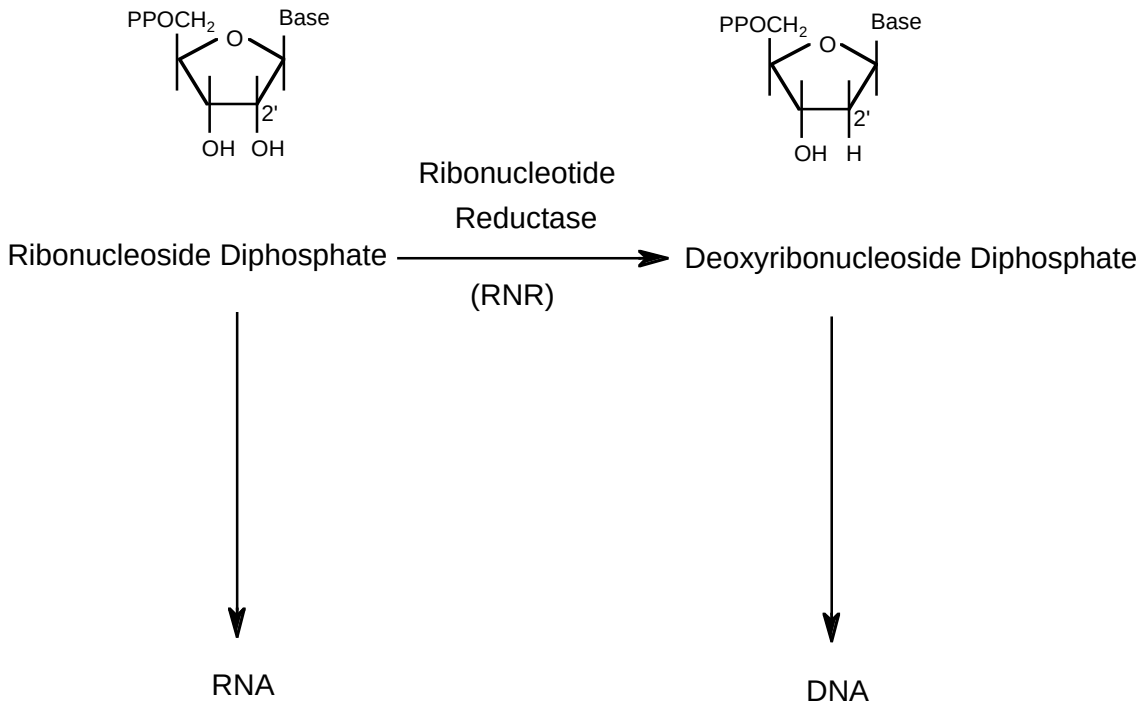


Figure 1. Ribonucleotide reductase - a key enzyme between RNA synthesis and DNA synthesis

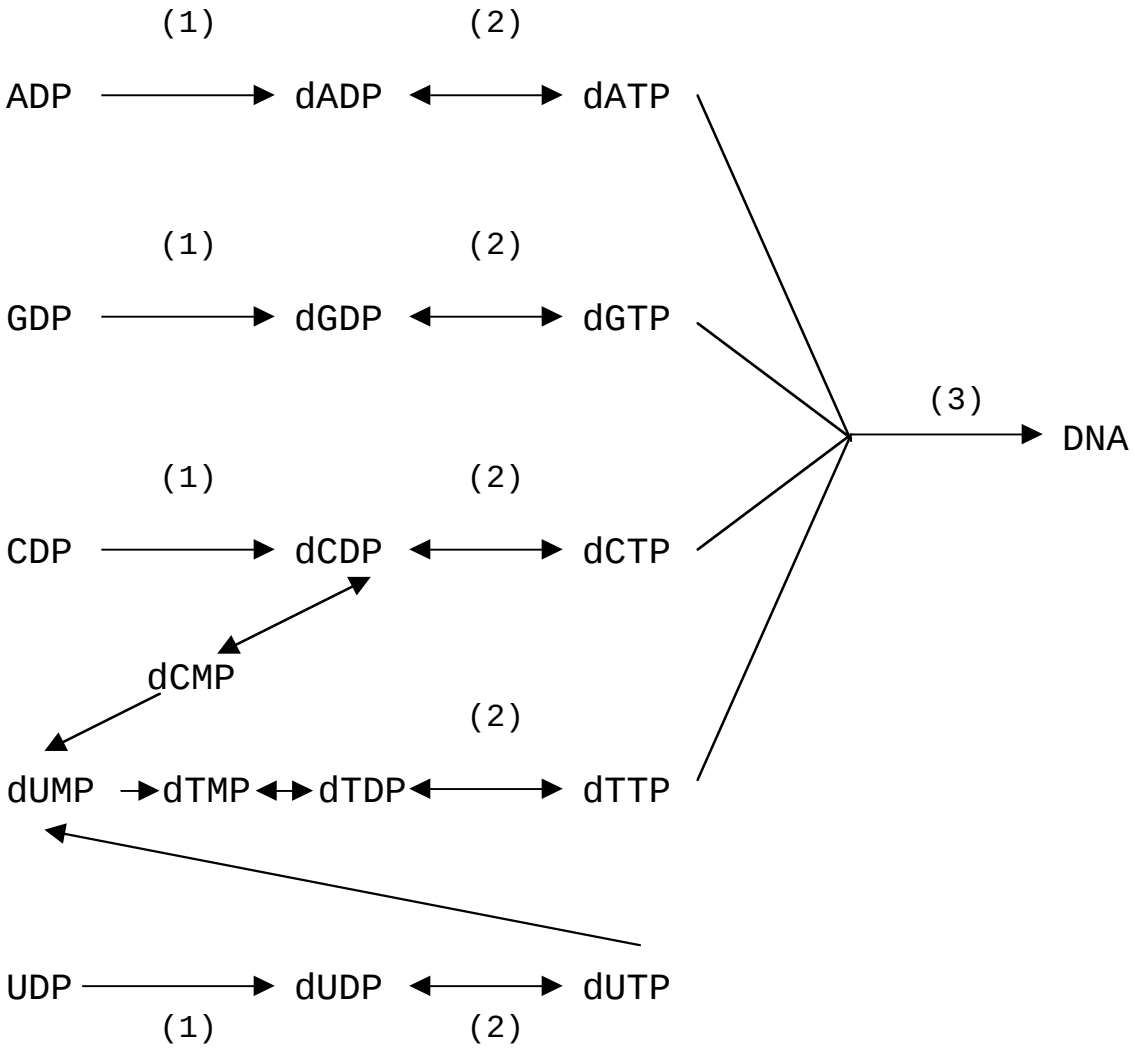


Figure 2. The DNA synthesis pathway from ribonucleotide reduction to DNA polymerization in *E.coli* and in eukaryotes

(1) Ribonucleotide reductase

(2) Nucleoside diphosphate kinase

(3) DNA polymerase

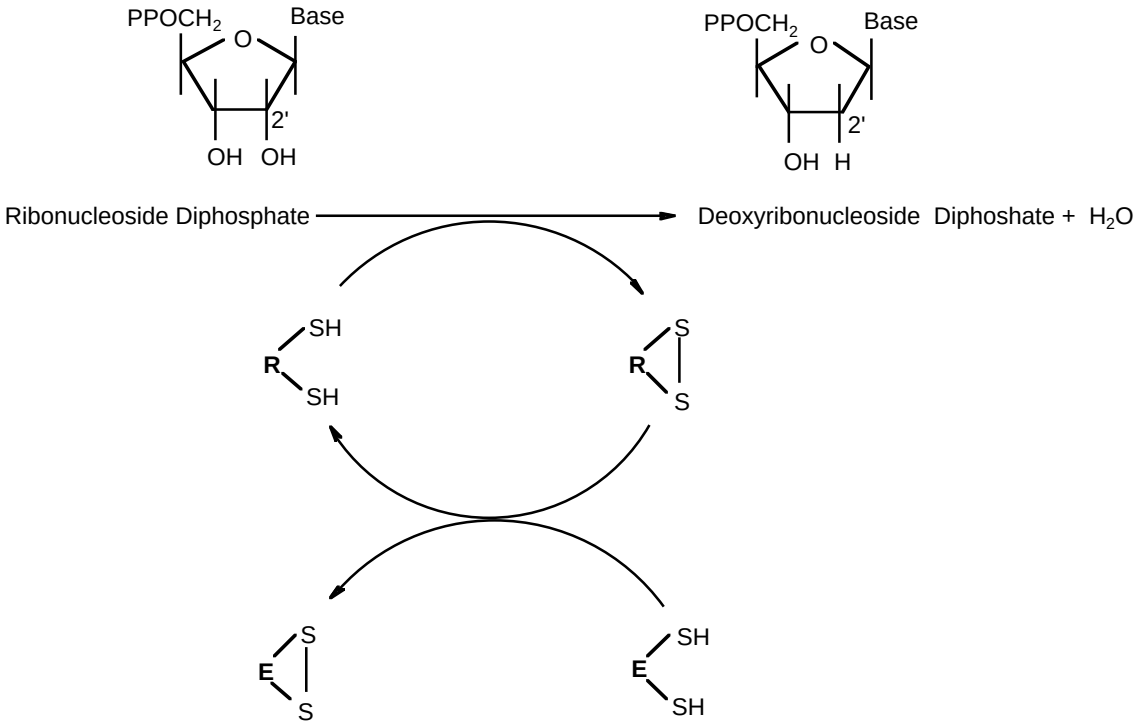
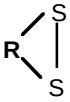
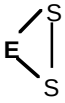


Figure 3. The catalytic reaction of ribonucleotide reductase

 = reduced form of ribonucleotide reductase

 = oxidized form of ribonucleotide reductase

 = oxidized form of another redox enzyme

 = reduced form of another redox enzyme

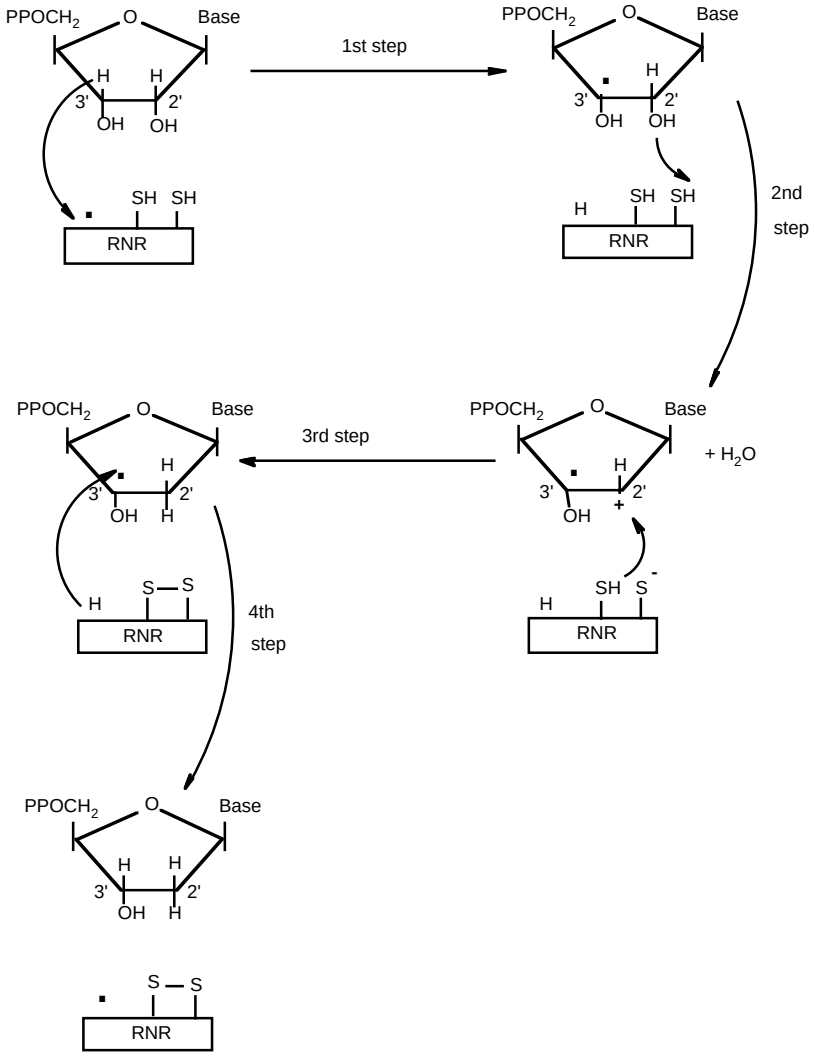


Figure 4. The proposed reaction mechanism of ribonucleotide reductase

Classes of ribonucleotide reductases

According to their cofactors and subunit composition, known RNRs are grouped into four classes (Table 1) (Stubbe and van der Donk 1995; Fontecave 1998) (Jordan and Reichard 1998). The cofactors generate protein free radicals, which are essential for ribonucleotide reduction. Despite their similar role in DNA synthesis and similar catalytic reactions, RNRs in different classes are highly diverse in cofactors, subunit composition and amino acid sequences.

In Class I RNR, the cofactor is two iron ions connected by an oxygen atom, which generates a tyrosine free radical. The enzyme complex consists of two identical large subunits and two identical small subunits. Class I RNR is in eukaryotes (Table 2), aerobic bacteria such as aerobic *E.coli* (Reichard 1988), herpes simplex virus (Averett et al. 1983) and vaccinia virus (Slabaugh and Mathews 1984).

In Class II RNR, the cofactor is coenzyme B_{12}, which generates a cysteine free radical (Licht et al. 1996). The enzyme consists of one subunit. Class II RNR is in aerobic and anaerobic bacteria such as *Lactobacillus* (Blakley 1978) and *Rhizobium* (Cowles et al. 1969).

In Class III RNR, the cofactor is a FeS cluster, which generates a glycine free radical (Mulliez et al. 1993). Like Class I RNR, Class III RNR consists of two identical large subunits and two identical small subunits. Unlike Class I RNR, Class III RNR is oxygen-sensitive. Class III

RNR is in strict and facultative anaerobic bacteria such as anaerobic *E.coli* (Fontecave et al. 1989) and methanogens (Reichard 1997). It is also in bacteriophage T4 (Young et al. 1994).

In Class IV RNR, the cofactor is two manganese ions connected by an oxygen atom (Willing et al. 1988). The possible protein free radical is unknown. Class IV RNR consists of two large subunits and one small subunit. Class IV RNR is in *Brevibacterium ammoniagenes*.

Some organisms have more than one class of RNR. For example, *E.coli* has Class I RNR for aerobic growth and Class III RNR for anaerobic growth. *Pseudomonas* has Class I and II RNR, and may also have Class III RNR (Jordan et al. 1999).