# This student paper was written as an assignment in the graduate course

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# Ribonucleotide Reductase – an enzyme that catalyzes free radical reactions

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# Ribonucleotide Reductase - outline

- What is it?
- Why is it unique?
- What reaction does it catalyze?
- What is its structure and how to detect it?

# Ribonucleotide Reductase (RNR)

- ribonucleoside-diphosphate reductase

  $NDPs + NADPH + H^+ \leftrightarrows dNDPs + NADP^+ + H_2O$

- ribonucleoside-triphosphate reductase

  $NTPs + NADPH + H^+ \leftrightarrows dNTPs + NADP^+ + H_2O$

  - NDPs: ribonucleoside diphosphates
  - dNDPs: deoxyribonucleoside diphosphates
  - NTPs: ribonucleoside triphosphates
  - dNTPs: deoxyribonucleoside triphosphates

# Ribonucleoside Diphosphate Reductase

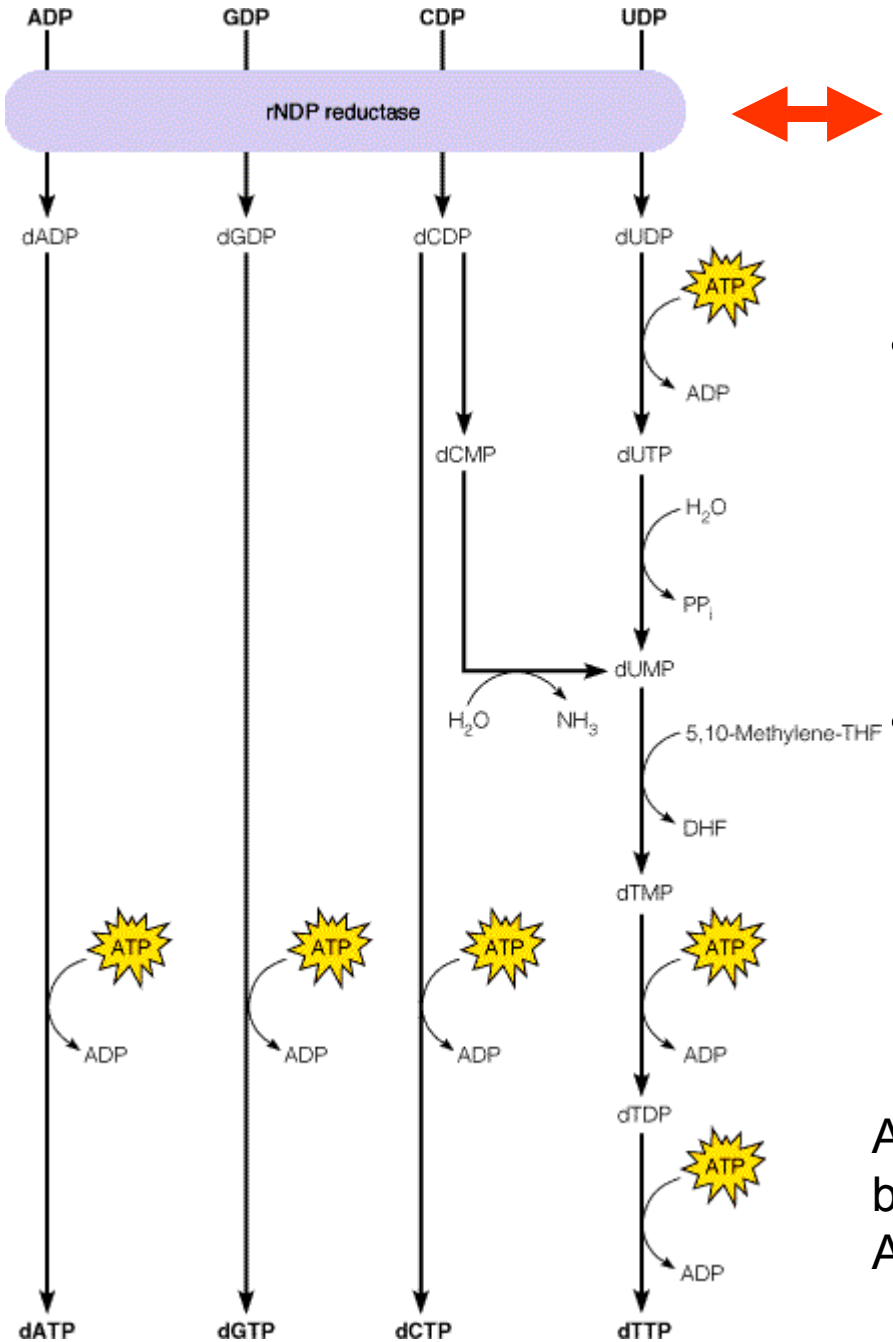
- An enzyme of the oxidoreductase class that catalyzes the formation of 2'-deoxyribonucleotides from the corresponding ribonucleotides using NADPH as the ultimate electron donor.
- The deoxyribonucleoside diphosphates are used in DNA synthesis.
  - Adapted from MeSH at NCBI

# Ribonucleoside Diphosphate Reductase - SYNONYMS

- 2'-deoxyribonucleoside-diphosphate:oxidized-thioredoxin 2'-oxidoreductase
- ADP reductase
- CDP reductase
- nucleoside diphosphate reductase
- reductase, ribonucleoside diphosphate
- ribonucleoside 5'-diphosphate reductase
- ribonucleoside diphosphate reductase
- ribonucleotide diphosphate reductase
- ribonucleotide reductase
- UDP reductase

# Why is RNR unique?

- The reduction occurs at a nonactivated carbon; no closely analogous chemical reactions are known.
- A free-radical mechanism is involved in the reaction.
- It catalyzes the rate-determining step in DNA precursor biosynthesis
  - *Chem Rev*. **98**:705-762.

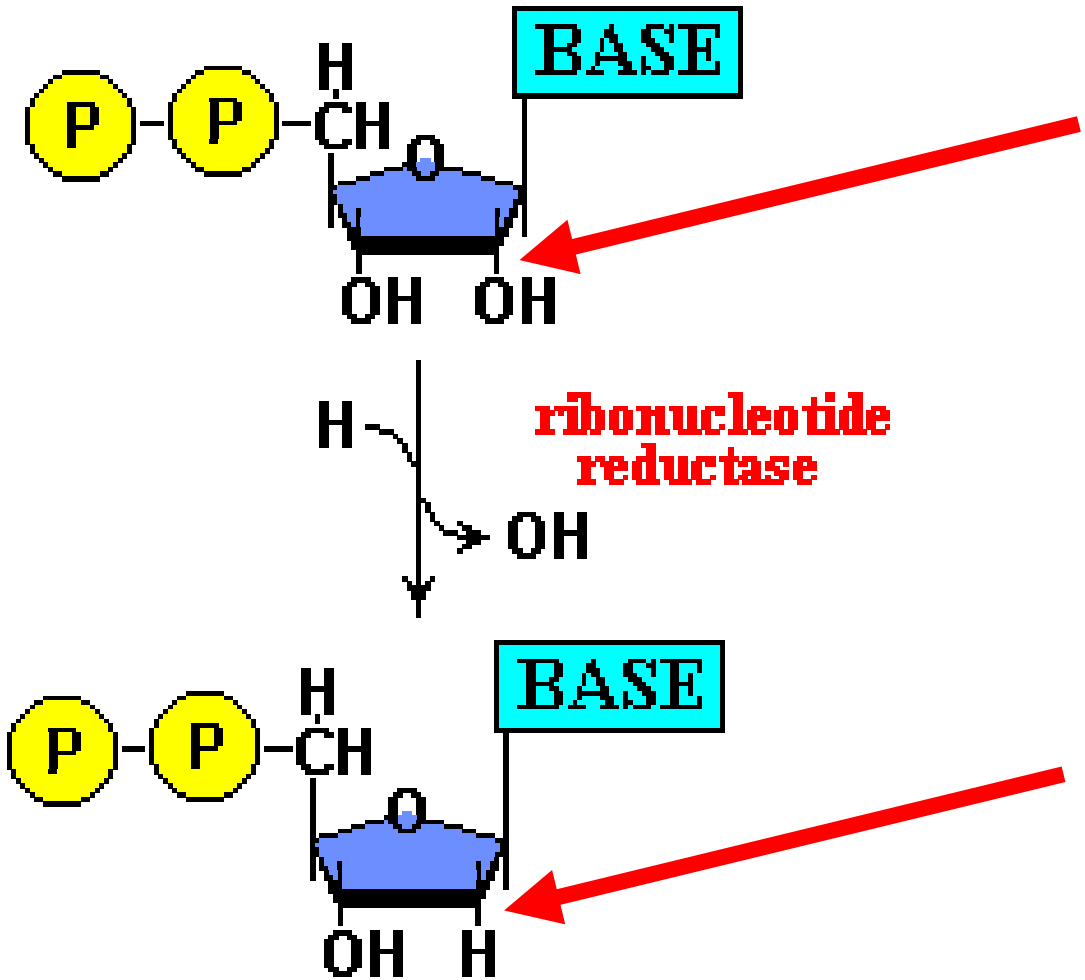



ribonucleoside-diphosphate reductase

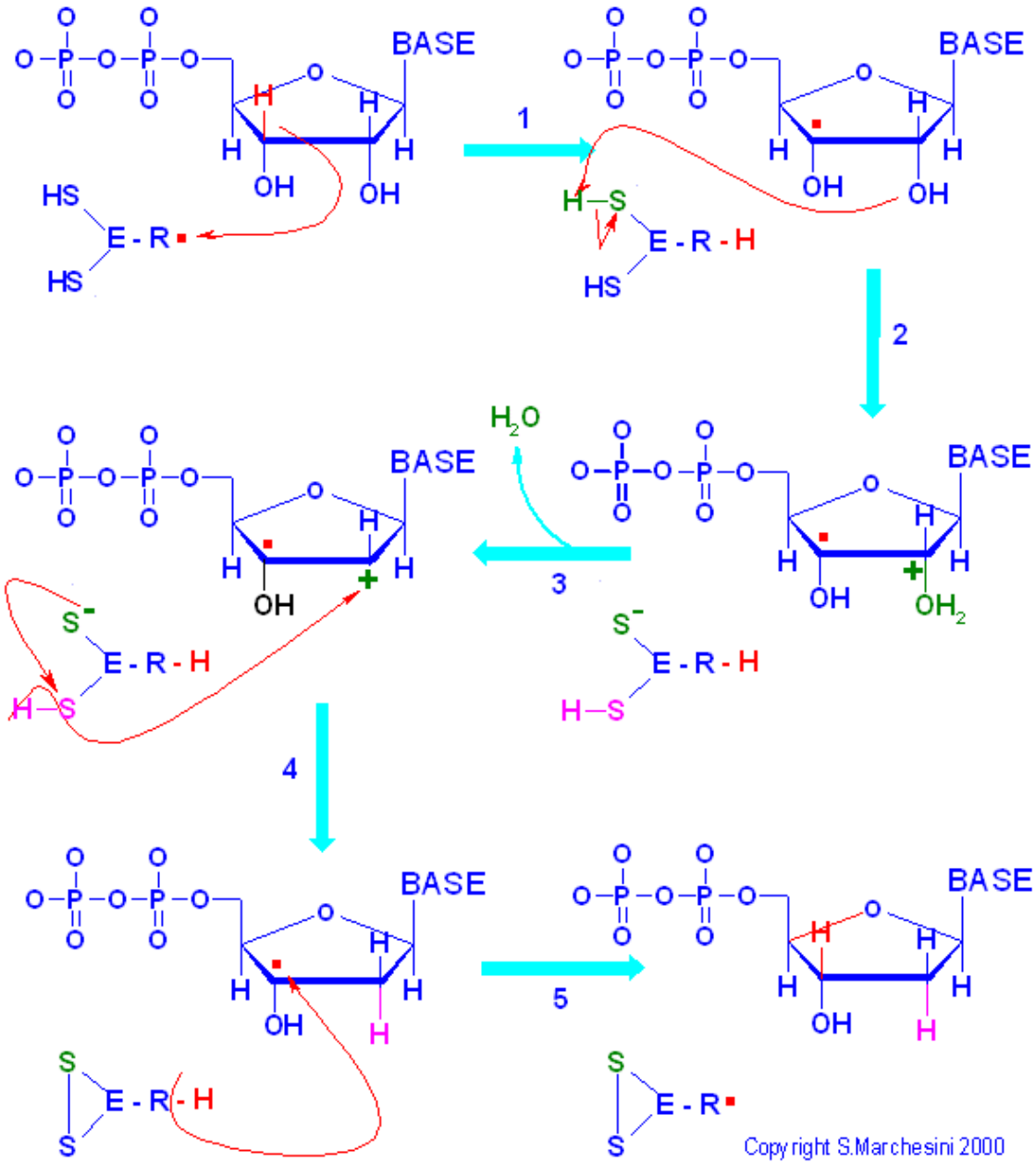
- Ribonucleoside-diphosphate reductase catalyzes NDPs into dNDPs
- dNDPs are further catalyzed to dNTPs which are DNA precursors.

Adapted from http://www.aw-bc.com/mathews/ch22/fi22p12.htm Accessed on 3/23/2005.

# The reductase (deoxy) reaction

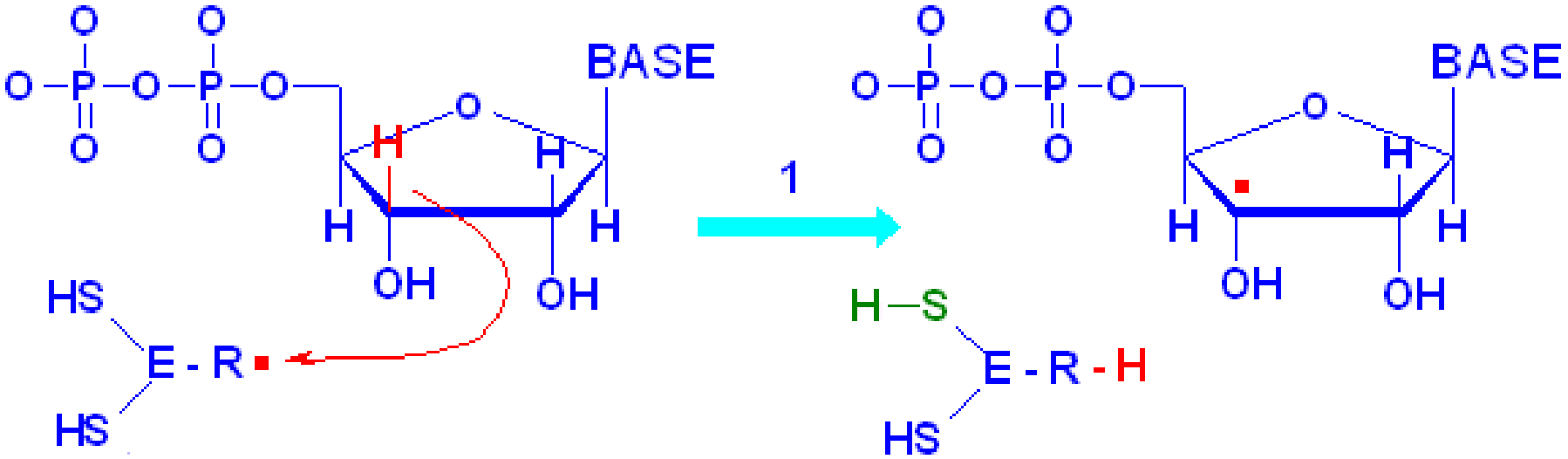



Adapted from http://www.people.virginia.edu/~rjh9u/ribreductase.html
Accessed on 3/23/2005.



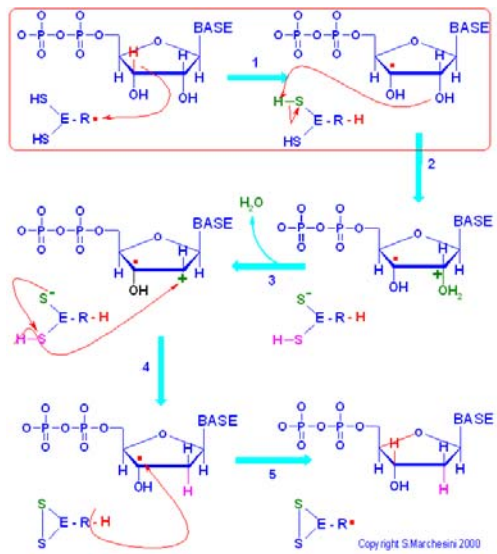

- The overview of reduction reaction at carbon 2' atom of ribonucleotide.
- A radical intermediate at carbon 3' is formed.
- The hydroxyl group at carbon 2' atom is reduced to hydrogen.

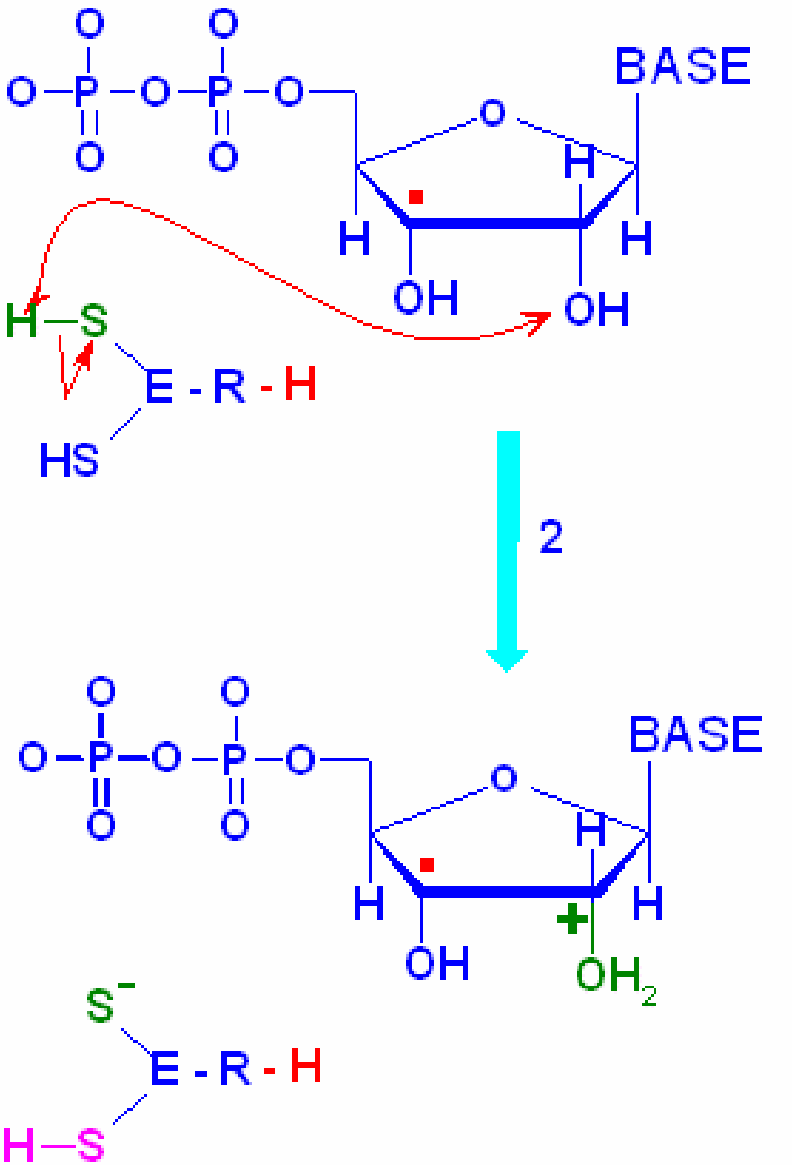
Adapted from http://www.med.unibs.it/~marchesi/ndp_reductase.html Accessed on 3/23/2005.



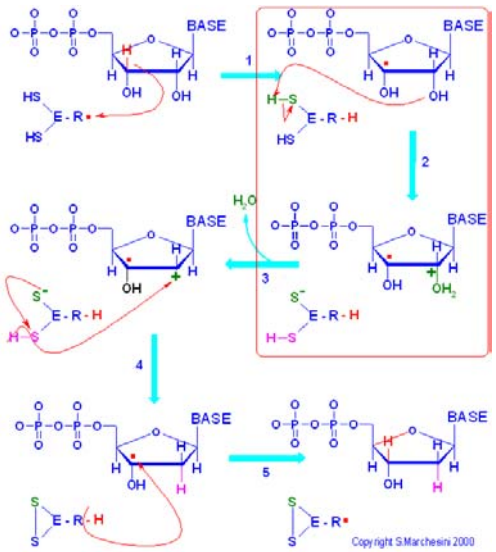

The free radical of ribonucleotide reductase abstracts a hydrogen atom from carbon 3' of the substrate, generating a free radical on the substrate.

Adapted from http://www.med.unibs.it/~marchesi/ndp_reductase.html Accessed on 3/23/2005.






One of the thiol groups of the enzyme donates a proton to  oxygen on C2'.




Adapted from http://www.med.unibs.it/~marchesi/ndp_reductase.html
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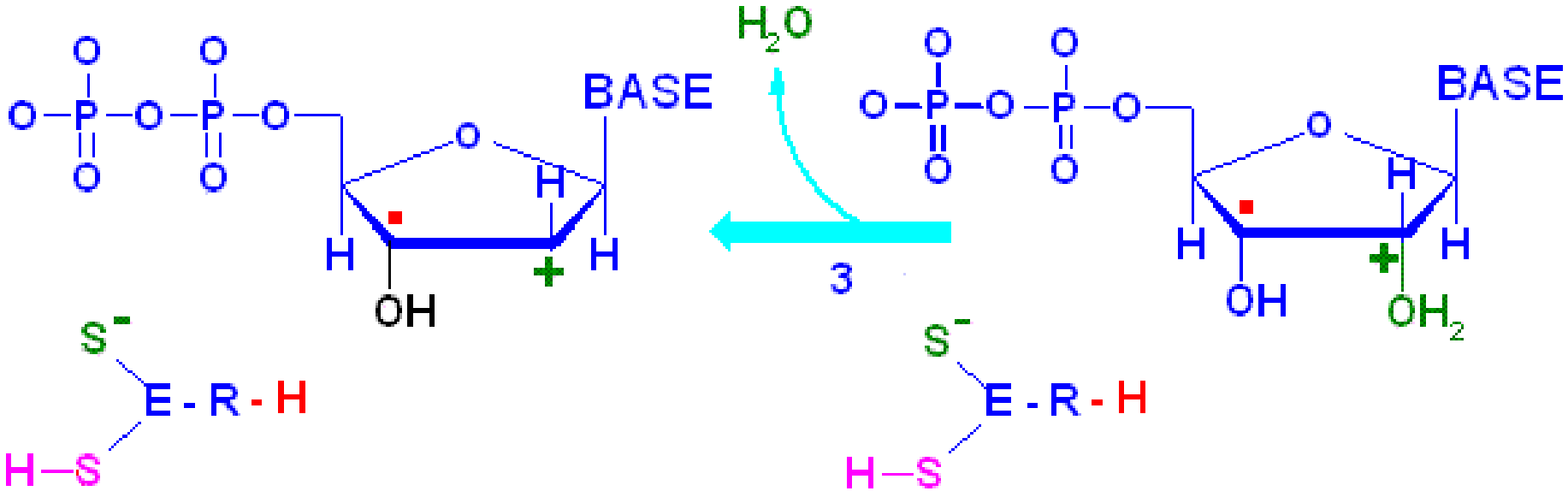
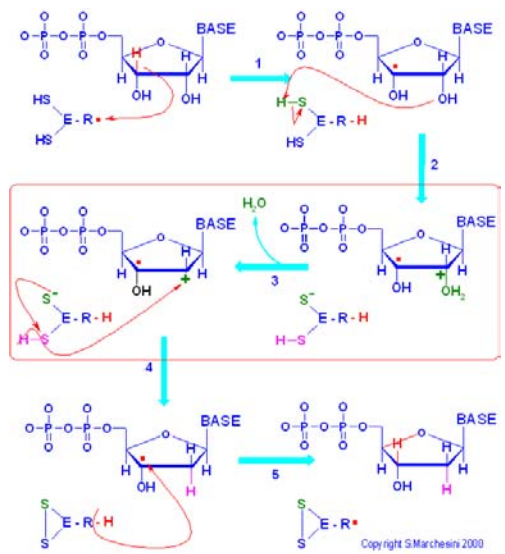
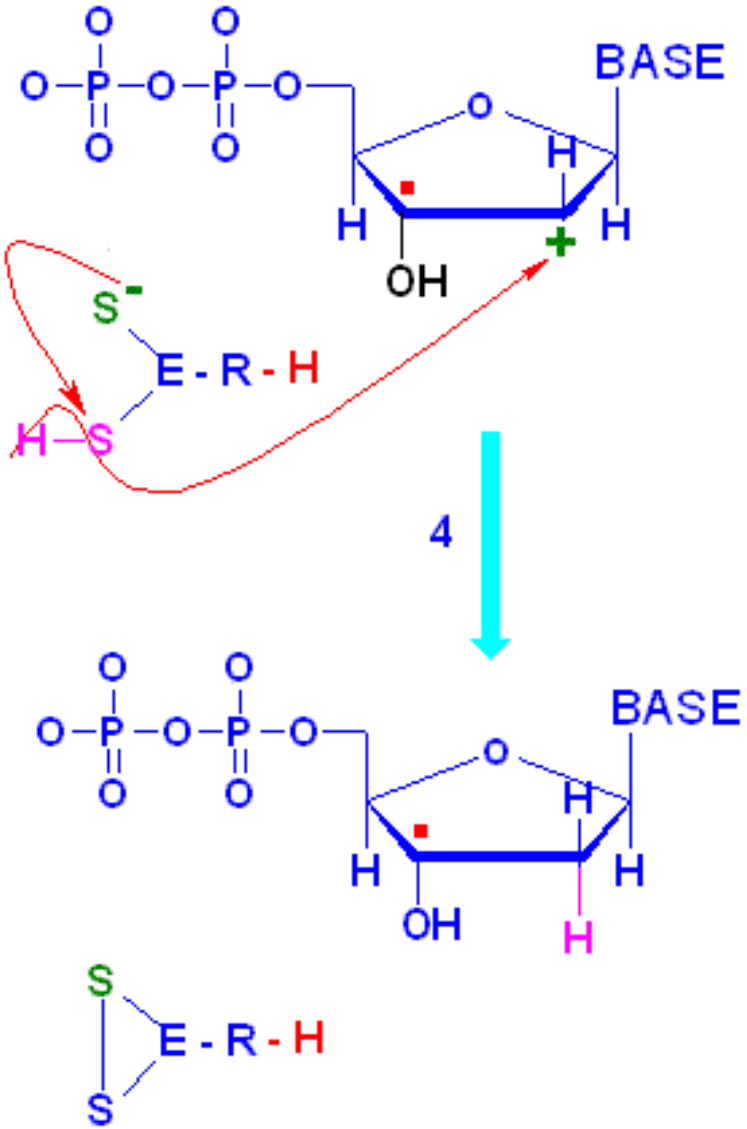


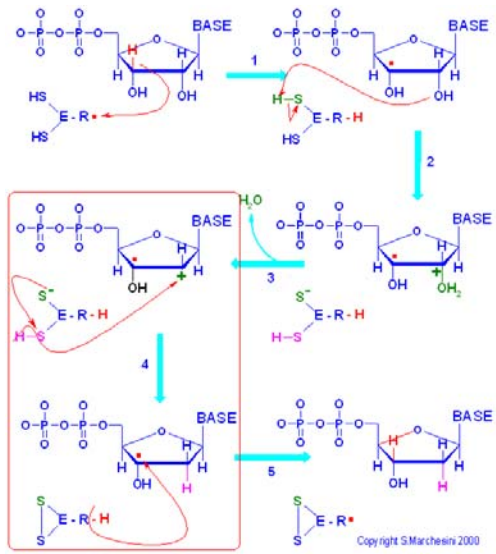

A water molecule is eliminated and a carbocation on C2' is produced.

Adapted from http://www.med.unibs.it/~marchesi/ndp_reductase.html
Accessed on 3/23/2005.






The carbocation on C2' is reduced by the second sufhydryl group.

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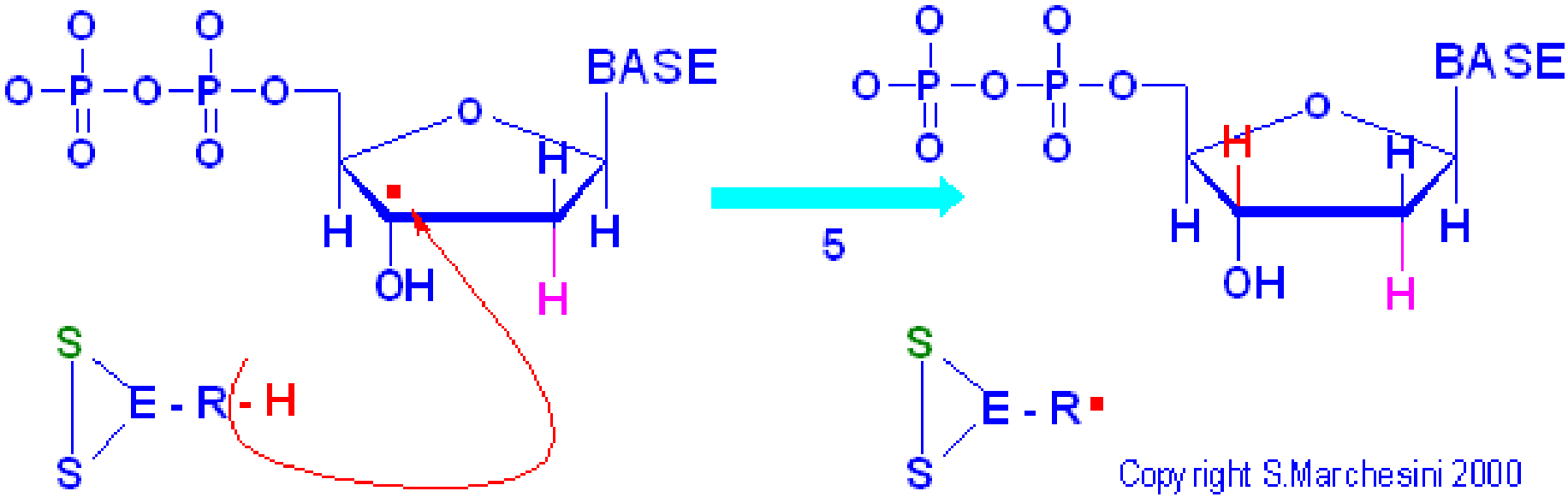
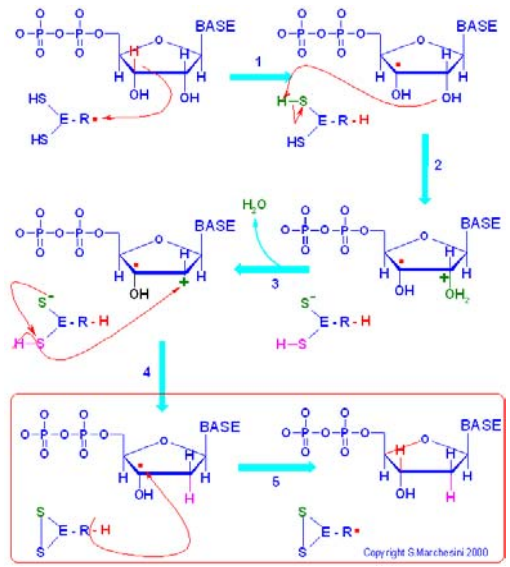
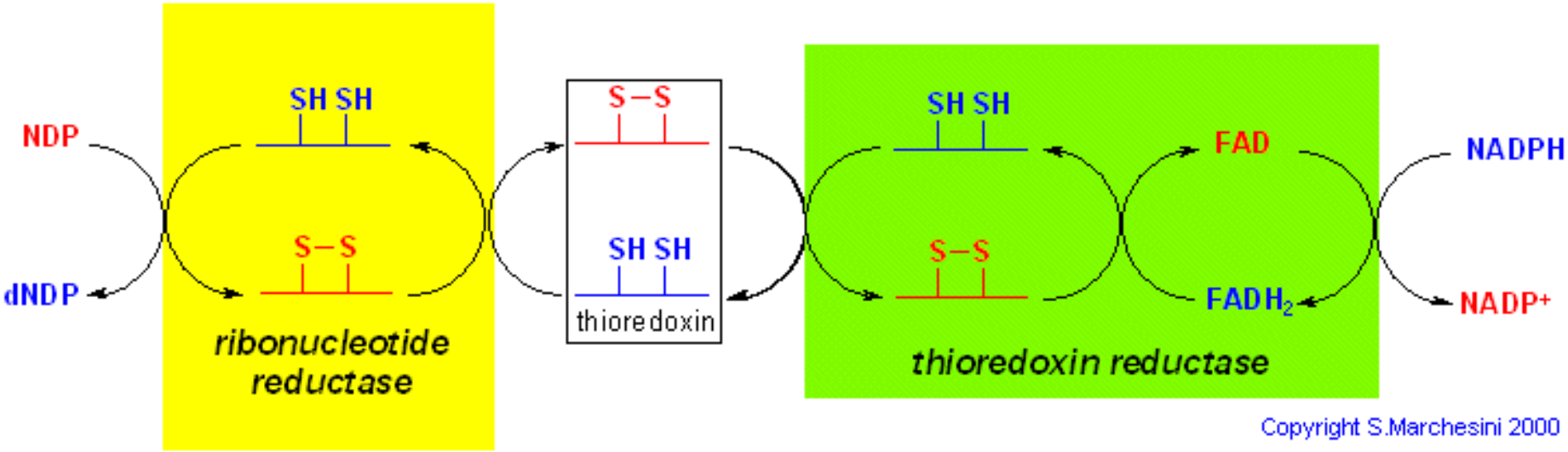



The enzyme donates a hydrogen atom to radical C3' to form the deoxyribonucleotide; the enzyme in converted in its radical form and must be reduced to its starting disulfhydryl form.




Adapted from http://www.med.unibs.it/~marchesi/ndp_reductase.html Accessed on 3/23/2005.

# The ultimate source of the electrons is NADPH




- The oxidized RNR is reduced in turn, by either thioredoxin or glutaredoxin.

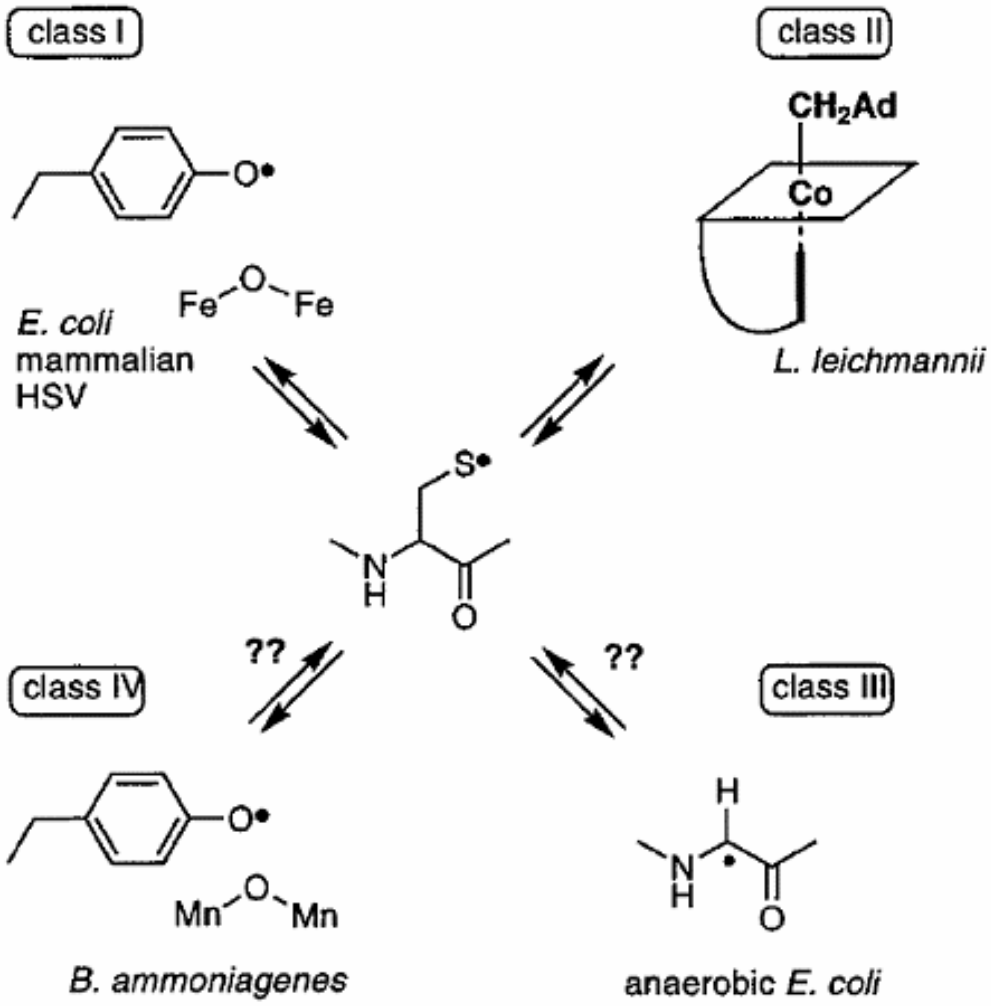
Adapted from http://www.med.unibs.it/~marchesi/nucmetab.html#RR
Accessed on 3/23/2005.

# Four classes of groups provide the initial radical

- Four classes of the active-site radical in the RNR have been reported.
- Class I: tyrosyl radical
- Class II: adenosyl cobalamin
- Class III: glycyl radical
- Class IV: Mn and tyrosyl radical

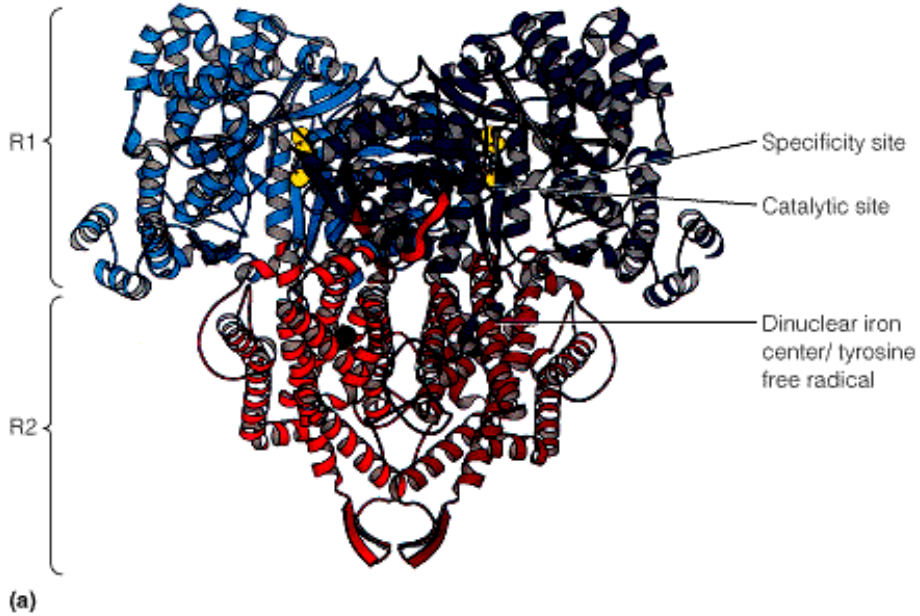
  – *Chem Rev*. **98**:705-762

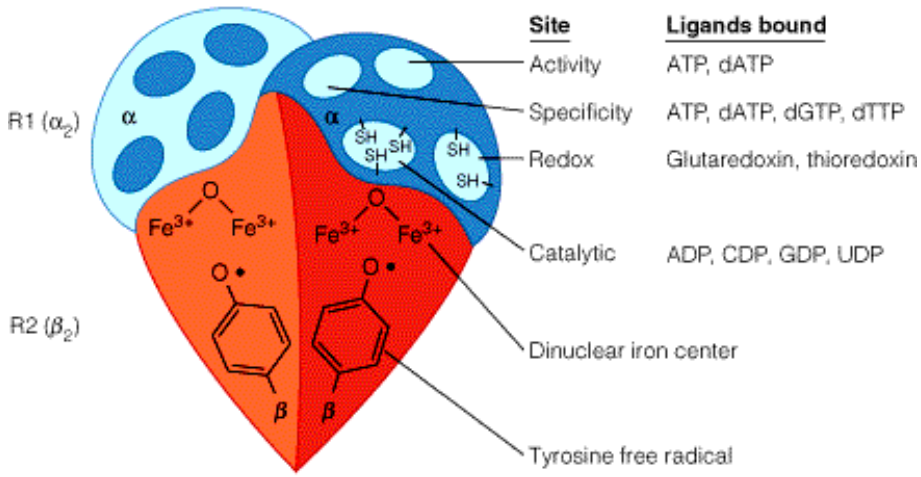
# All of the 4 classes generate a thiyl radical that initiates nucleotide reduction.




*Chem Rev*. **98**:705-762

# The structure of ribonucleotide reductase (Class I)




The most common form of **ribonucleotide reductase** (Class I) is an dimer.

From Uhlin U and Eklund H. (1994) *Nature* 370:533-539.

# RNR Tyrosyl radical and Co(II) detected by EPR

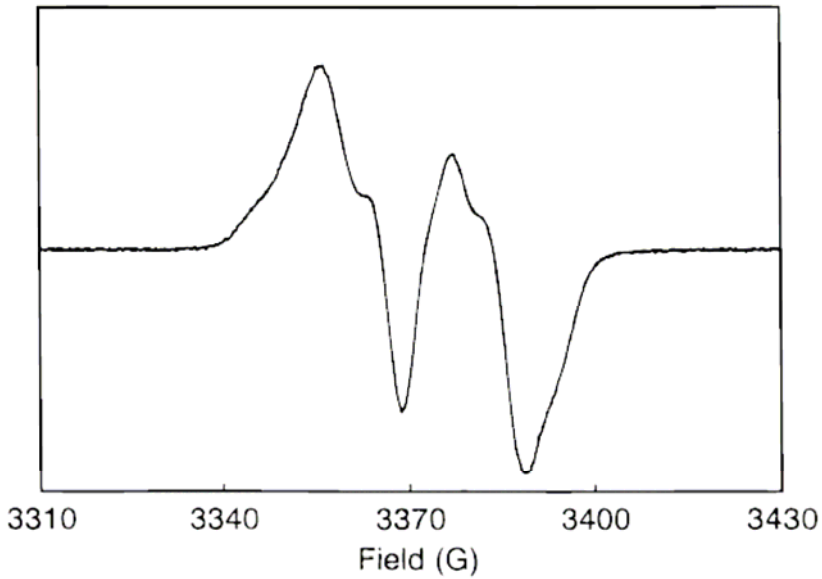



**Figure 3.** EPR spectrum of the tyrosyl radical in ribonucleotide reductase from *E. coli*. Conditions: microwave frequency, 9.428 GHz; temperature, 20 K; power, 10 $\mu$W, modulation amplitude, 4 G; modulation frequency, 100 kHz; time constant, 0.126 s.

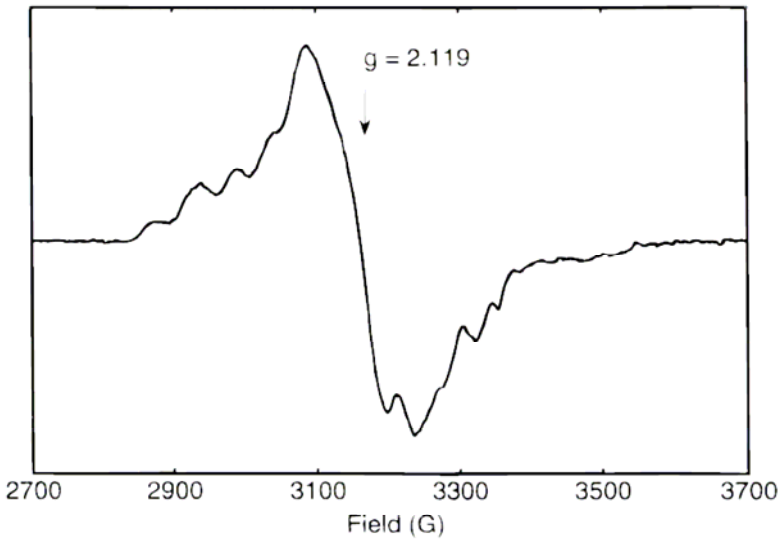



**Figure 10.** EPR spectrum of cob(II)alamin exchange coupled to the thiyl radical on Cys408 in class II RNR from *L. leichmannii*. Conditions: temperature, 100 K; microwave frequency, 9.41 GHz; microwave power, 10 mW; modulation frequency 100 kHz; modulation amplitude, 4 G; time constant, 1.3 s; and scan time 671 s.[18]

*Chem Rev*. **98**:705-762

# Summary

- **RNR catalyzes the reduction of ribonucleotides to deoxyribonucleotides**
- **This reduction reaction uses thiyl radical as active group to transfer electrons from NADPH, through glutaredoxin or thioredoxin to ribonucleotides.**
- **RNR determines the rate of DNA precursors synthesis.**