Ribonucleotide reductases and their occurrence in microorganisms: A link to the RNA/DNA transition

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Abstract: The evolution of a deoxyribonucleotide synthesizing ribonucleotide reductase might have initiated the transition from the ancient RNA world into the prevailing DNA world. At least five classes of ribonucleotide reductases have evolved. The ancient enzyme has not been identified. A reconstruction of the first ribonucleotide reductase requires knowledge of contemporary enzymes and of microbial evolution. Experimental work on the former focuses on few organisms, whereas the latter is now well understood on the basis of ribosomal RNA sequences. Deoxyribonucleotide formation has not been investigated in many evolutionary important microorganisms. This review covers our knowledge on deoxyribonucleotide synthesis in microorganisms and the distribution of ribonucleotide reductases in nature. Ecological constraints on enzyme evolution and knowledge deficiencies emerge from complete coverage of the phylogenetic groups.

Introduction

Ribonucleotide reductases (RNRs) are known as essential enzymes providing deoxyribonucleotides for DNA replication. They are also evolutionary key enzymes. The invention of a ribonucleotide reductase made the transition from a RNA world to a DNA world possible [1,2]. Intensive research on abiotic formation of biomolecules has not revealed a chemical route to deoxyribonucleotides [3–5].

Our knowledge of complex and well-organized catalytic RNA systems raises the likelihood that the first ribonucleotide reducing enzyme was either a ribozyme or a ribonucleoprotein. Five different classes of RNRs are known in the modern world, and even more may await discovery. None of these can currently be identified as the ancient RNR. Therefore, it was proposed that all arose by protein-for-RNA replacement events [6]. Ribonucleotide reduction, however, has not been studied in many evolutionary important microorganisms.

Future research can contribute to an understanding of the emergence of DNA, if it combines our understanding of bacterial evolution with a broad knowledge of contemporary RNRs. The former has been established based on ribosomal RNA sequences [7,8], the latter has progressed extensively since the last review on the distribu-

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tion of RNRs in nature 10 years ago [9]. The time seems ripe for a review of our current knowledge on deoxyribonucleotide formation in microorganisms and the distribution of ribonucleotide reductases in nature. Salvage supply of DNA precursors, the alternative to de novo synthesis, is considered, but the biochemistry of deoxyribonucleotide modification, e.g. thymidylate synthesis, is excluded. In this review I will first briefly describe the chemistry of ribonucleotide reduction and the enzyme classes. The main part describes deoxyribonucleotide formation in phylogenetic groups, covering the domains Archaea, Bacteria and Eucarya. Finally, I will discuss ecological and evolutionary features of ribonucleotide reductases.

De novo and salvage formation of deoxyribonucleotides

Ribonucleotide reductases are radical enzymes. They embody essential radical sites which during catalysis accommodate a hydrogen atom abstracted from the substrate. Reduction of the activated substrate, a ribonucleotide containing a sugar-based free radical, involves redox active cysteines. The enzymes reduce all four ribonucleotides to the corresponding deoxyribonucleotides. Enzyme activity and substrate specificity are regulated by ATP and dNTPs acting allosterically. The nucleotide binding sites are located together with the cysteine domains on one protein, R1. The radical site has the properties of a cofactor and defines the different enzyme classes.

All RNRs investigated use a common reaction pathway for ribonucleotide reduction (Fig. 1) [10,11]. Substrate binding to the reduced enzyme induces activation of the radical domain and hydrogen abstraction from the 3'-carbon of the sugar moiety. Reduced cysteines mediate the 2'-hydroxyl reduction with retention of configuration. The intermediate, energetically unfavourable 2'-carbocation is stabilized by interaction with the 3'-carbon radical. Finally, the hydrogen atom is transferred from the radical domain to the product radical.

All RNRs have two functional domains (Fig. 2). R1 contains binding sites for allosteric and substrate nucleotides and redox active cysteines [12]. The complex allosteric regulation by ATP
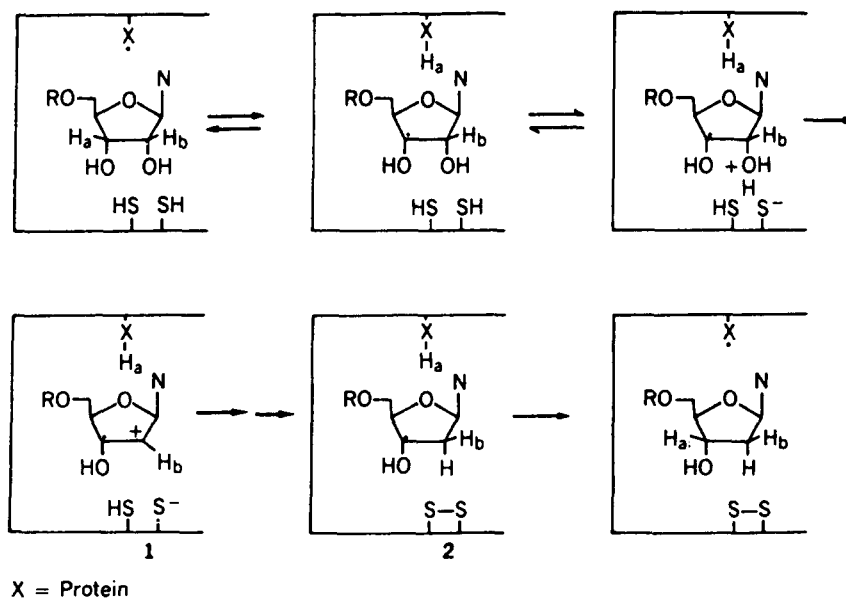

X = Protein

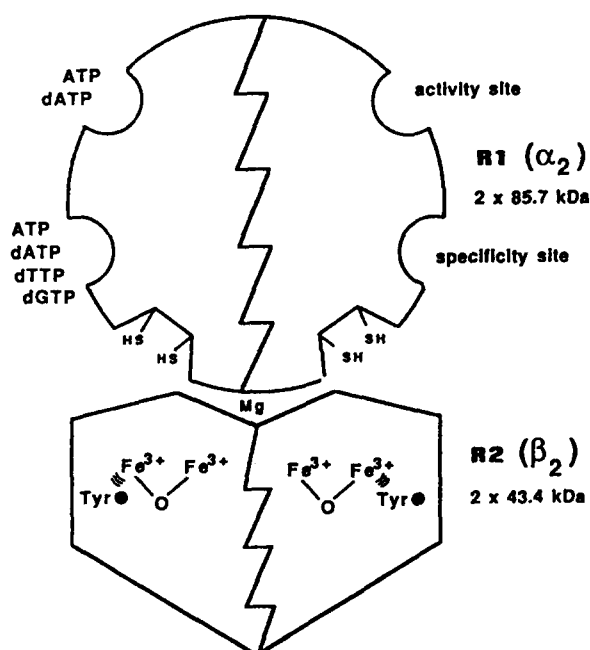Fig. 1. Proposed reaction mechanism for ribonucleotide reductases (from [11]).

Fig. 2. Model of *E. coli* ribonucleoside diphosphate reductase (from [12]).

together with the R1 domain on one polypeptide chain [28–30].

The demand for high accuracy of DNA replication favours de novo synthesis of dNTPs over salvage supply. Deoxyribonucleoside and deoxyribonucleoside monophosphate kinases are often not present or have only a low synthesis rate, with exception of thymidylate kinases. Salvage synthesis of DNA precursors seems to be restricted to organisms living in a nutrient-rich habitat, e.g. *Giardia lamblia* and *Lactobacillus* sp.

Salvage activities, however, can disturb the determination of RNR activity in crude extracts. Enzyme assays are performed with radioactively labelled ribonucleotides. After incubation all nucleotides are dephosphorylated and the deoxynucleosides formed are determined by chromatography [27,31]. Phosphatases and phosphorylases can form radioactive deoxynucleosides from the substrate and deoxynucleosides present in crude extracts, thus mimicking RNR activity [32,33]. This problem is circumvented in enzyme assays which detect the reaction product as deoxyribonucleotide [34–37].

Nature of ribonucleotide reductases

Oxygen-dependent enzymes

Oxygen is nowadays easily available and has the required high oxidation potential to abstract a hydrogen atom from a non-activated hydrogen–carbon bond. It is widely used in nature to initiate the degradation of recalcitrant substances, e.g. lignin, aromatic compounds and hydrocarbons. In all these cases hydrogen atom abstraction from the substrate is irreversible. But ribonucleotide reduction requires a reversible C-H activation. This difference may explain why oxygen is only used for the oxidative activation of radical domains in class I and III enzymes [27,38,39].

The active enzymes are sensitive towards reducing reagents which can penetrate the protein shield around the radical domain. Hydroxyurea (HU) was found to be a selective inhibitor of oxygen-dependent RNRs [40,41]. DNA synthesis ceases, but RNA and protein synthesis are not

and all four dNTPs provides a balanced supply of the precursors for DNA synthesis [13,14]. Constant intracellular dNTP pools are crucial for a low mutation rate during DNA replication [15]. The reducing equivalents are transferred from NADPH via either thioredoxin, glutaredoxin or unknown proteins to a pair of cysteines on the surface of R1 [16,17]. This pair of cysteines is located near the C-terminus in class I RNRs. Internal redox active cysteines in/near the substrate binding site are either involved in the 2'-hydroxyl reduction or serve as a radical relay in the 3'-hydrogen abstraction [18–23].

The nature of the cofactor, a radical domain, characterizes the different classes of RNRs. The small protein subunit R2 of class I enzymes contains μ-oxo bridged binuclear Fe(III) clusters and tyrosyl radical(s) [12,24]. Deoxyadenosylcobalamin is the radical precursor in class II RNRs [25,26]. Class III enzymes have a small subunit R2 with a highly oxidized manganese cluster [27]. Recent studies suggest that a glycine radical is present in class IV proteins which is located

impaired. The cell cycle is blocked in early S-phase. Growth rates of bacteria and eukaryotic cells possessing a class I or III RNR are usually reduced to 50% at 1–2 mM HU, and DNA replication and cell division ceases completely above 10 mM HU. The inhibition can often be suppressed by a supply of deoxyribonucleotides or deoxyribonucleosides in the medium. This indicates the specificity of HU inhibition, but it requires the presence of salvage kinases. Hydroxyurea can chelate metal ions with the hydroxamate group and undergoes one-electron oxidations. High concentrations (≥ 10 mM) induce metal ion limitations and reductive stress. The specific inhibition of DNA synthesis occurs at lower concentrations of the drug. Proliferation cessation could in only one case not be traced to a RNR: HU inhibits chlorophyll biosynthesis in *Euglena* [42].

Class I: iron(III)-tyrosyl radical proteins

The aerobic ribonucleoside diphosphate reductase (*nrdAB*) from *Escherichia coli* is certainly the best studied RNR [43]. The crystal structure of the small subunit R2 has recently been solved [44,51]. It has an $\alpha_2\beta_2$ structure (Fig. 2). Several excellent reviews describe structural and functional aspects of the enzyme [11,12,45–47].

Current studies focus on the mechanism of the activation reaction and the nature of the hydrogen abstraction pathway [24,39,47,48]. The R2 dimer harbours the radical domains, deep inside the protein, each consisting of a μ-oxo bridged binuclear iron(III) center and a tyrosyl radical nearby. The radical is at least 10 Ångström away from the surface of R2. A long-range transport of one electron has to occur between the tyrosyl radical and the 3'-position of the substrate bound to R1. The C-terminal nonapeptide of R2 binds the small subunit to R1 and recent studies suggest that this part of R2 and redox active cysteines from R1 participate in the electron transfer [20,21,49,50].

The nucleotide sequences of several class I RNRs have been determined, e.g. enzymes from *E. coli*, T4 virus, mouse, clam, yeast and several herpes viruses [47,49,51]. *NrdA* from *E. coli* en-

codes a 761-amino acid polypeptide. The first 200 residues are not conserved in herpes R1 genes where a protein kinase activity is located on the N-terminal region. Forty-five residues are conserved in R1 genes, whereas 18 from 375 amino acids are invariant between R2 genes. The binding sequence of the R2 C-terminus is species-specific. This allows the development of new antiviral drugs inhibiting subunit association.

An open question concerns the distribution of class I RNRs in other bacteria. The phylogenetic tree indicates that the main bacterial groups evolved early before the appearance of free atmospheric oxygen. Aerobic bacteria sensitive towards hydroxyurea appear in several branches, but detailed studies of enzyme structures are lacking, except for *E. coli* and *Corynebacterium ammoniagenes*.

Class III: manganese proteins

Unbalanced growth and arrest of DNA synthesis in coryneform bacteria is essential for the commercial production of ribonucleotides and is caused by manganese limitation. The requirement could be traced to a RNR [52,53]. The manganese enzyme from *Corynebacterium ammoniagenes* (formerly *Brevibacterium ammoniagenes*) has been purified and characterized [27,54]. It resembles in the overall structure class I RNRs. The small subunit R2 contains manganese instead of iron ions. Absorption and EPR spectra suggest the presence of high-valency μ-oxo bridged manganese(III,IV) complexes. The enzyme is hydroxyurea-sensitive and requires molecular oxygen for reactivation. A free radical has not been detected. The manganese complex itself may serve as radical domain in this enzyme. A manganese cluster is used in the water-oxidizing enzyme in chloroplasts [55]. High-valency manganese complexes are easily formed in a spontaneous reaction from manganese(II), molecular oxygen and carboxylates, and they are sensitive towards reduction [56].

Oxygen-independent enzymes

Many anaerobic, facultative anaerobic and aerobic microorganisms possess a deoxyadenosyl-cobalamin-dependent RNR which is neither oxy-

gen-dependent nor oxygen-sensitive. None of the other known RNRs can function in aerobic and anaerobic environments.

Class II: cobalamin-dependent enzymes

Vitamin B_{12} or deoxyribonucleosides have earlier been identified as growth factors for lactic acid bacteria [57,58]. The presence of a B_{12}-dependent RNR was first demonstrated in *Lactobacillus leichmanii* [59,25]. The class II enzyme is not inhibited by hydroxyurea. The radical chemistry of cobalamin-dependent RNR differs slightly from all other B_{12}-dependent enzymes. Tritium from $[5'-^3H_2]$deoxyadenosylcobalamin [60] is not exchanged with the substrate under turnover conditions. It is released into water [61,62]. This points towards the existence of an acidic radical relay between the 3'-position and the deoxyadenosyl radical, perhaps one of the internal

redox active cysteines of R1 [63–66]. The specific exchange reaction has been used to detect B_{12}-dependent RNRs in several microorganisms (Table 1).

Oxygen-sensitive enzymes

Class IV: glycine radical proteins

The first strictly anaerobic ribonucleotide reductase was recently isolated and characterized from *E. coli* [67–71,28–30]. The enzyme is a homodimer of 154 kDa and contains in the active state iron–sulphur cluster(s) and glycine radical(s). The organic free radical is oxidatively introduced in an enzymatic activation reaction with concomitant reductive cleavage of *S*-adenosylmethionine into 5'-deoxyadenosine and methionine. This type of activation reaction was previously detected only with pyruvate–formate

Table 1

Cobalamin-dependent ribonucleotide reductases in microorganisms

| B_{12}-dependent RNR activity		Positive ^3H_2O release assay		Negative ^3H_2O release assay	
Bacillus megaterium	[138]	*Bacillus megaterium*	[138]	*Escherichia coli*	[61,89]
Corynebacterium nephridii	[130]	*Clostridium sticklandii*	[61,89]	*Aerobacter aerogenes*	[61]
Lactobacillus leichmannii	[132]	*Clostridium thermoaceticum*	[89]	*Agmenellum quadruplicatum*	[115,119]
Lactobacillus acidophilus	[132]	*Clostridium tetanomorphum*	[61,89]	*Methanobacillus omelianskii*	[89]
Rhizobium japonicum	[96]	*Corynebacterium nephridii*	[61,89]	*Pseudomonas*	[89]
Rhizobium leguminosarum	[96]	*Lactobacillus leichmannii*	[61]	*Micrococcus lysodeikticus*	[89]
Rhizobium meliloti	[96]	*Lactobacillus acidophilus*	[61]	*Synechococcus 7003*	[115]
Rhizobium phaseoli	[96]	*Pseudomonas stutzeri*	[89]	*(Coccochloris elabens)*	
Rhizobium trifolii	[96]	*Micrococcus denitrificans*	[89]		
Thermus X-1	[90]	*Sphaerophorus varius*	[89]		
Streptomyces aureofaciens	[131]	*Synechococcus* spp.	[115,116]		
		Thermus aquaticus	[89]		
		Anacystis nidulans	[115,116]		
		Coccochloris peniocystis	[116]		
		Nostoc commune	[116]		
		Anabaena spp.	[116]		
		Oscillatoria prolifera	[116]		
		Scytonema hofmanni	[116]		
		Fremyella diplosiphon	[116]		
		Plectonema boryanum	[116]		
		Phormidium luridum	[116]		
		Phormidium autumnale	[116]		
		Astasia longa	[89]	*Marchantia polymorphia*	[89]
				Cordyceps militaris	[89]
Euglena gracilis	[150]			*Ochromonas* spp.	[89]
Pithomyces chartarum	[201]	*Euglena gracilis*	[89,116]	*Chlorella* spp.	[116]
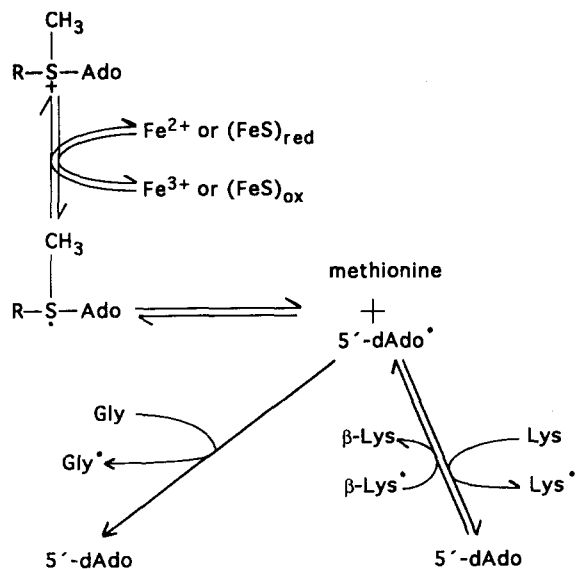
Fig. 3. Postulated radical enzymology of S-adenosylmethionine.

lyase from *E. coli* [72]. The gene *nrdD* is located at 96 minutes on the genome, as found by the Kohara library. The deduced amino acid sequence shows 72% similarity to SunY from coliphage T4, now a putative anaerobic RNR [29,73].

The radical chemistry of S-adenosylmethionine is also employed by nature in lysine 2,3-aminomutase from clostridia [74–78]. Activation of S-adenosylmethionine is initiated by one-electron reduction and generates an unstable sulphuranyl radical which, like other sulphuranyl radicals, may dissociate to methionine and a 5'-deoxyadenosyl radical (Fig. 3). The environment supplied by the proteins determines the fate of the organic free radical: it serves as a catalytic radical in lysine 2,3-aminomutases, whereas hydrogen abstraction from a specific glycine residue leads to enzyme activation of ribonucleotide reductase and pyruvate–formate lyase and formation of 5'-deoxyadenosine.

Class V: methanogenic RNRs

Initial studies of ribonucleotide reduction in *Methanobacterium thermoautotrophicum* have indicated that the enzyme from strict anaerobic methanogenic bacteria differs from all other

known classes [79,80]. The reductase activity is oxygen-sensitive and inhibited by S-adenosylmethionine and reducing agents. The structure of the radical domain is currently unknown.

Distribution of ribonucleotide reductases

Archaea

The domain Archaea includes methanobacteria, halobacteria, and sulphobacteria. Methano- and sulphobacteria have evolved very slowly and may represent the oldest bacteria. Sulphobacteria have many eukaryotic characters, a fact which has caused discussions on their phylogenetic position in rooted evolutionary trees [81–86]. Halobacteria have evolved significantly as indicated by ribosomal transversions [84]. They are adapted to aerobic life, but some strains are capable of anaerobic proliferation, gaining energy from respiration of fumarate, nitrate, dimethylsulphoxide or trimethylamine-N-oxide [87,88].

Deoxyribonucleotide formation has only been studied in methanogenic bacteria. *Methanobacterium thermoautotrophicum* and *Methanobacterium* M.o.H. (*'Methanobacillus omelanskii'*) do not contain a class II RNR [79,89]. Recent investigations have provided evidence that the *M. thermoautotrophicum* enzyme is the prototype of a new class of anaerobic RNR [79,80].

Bacteria

Ribonucleotide reduction has not been studied in many bacterial divisions. We lack the knowledge of deoxyribonucleotide formation in Thermotogales, Verrucomicrobium, Chloroflexaceae, Planctomyces, Bacteroides and Cytophaga, Chlorobiaceae and Spirochaetes.

Deinococci
Thermus aquaticus and *Thermus* X-1 react positive in the ^{3}H-release assay with [5'-^{3}H$_2$]deoxyadenosylcobalamin [89]. A monomeric ribonucleoside triphosphate reductase has been purified from *Thermus* X-1 [90]. The enzyme depends on cobalamin, has a temperature optimum of 70–

75°C and is allosterically controlled by nucleotides.

Chlamydiae

The genus *Chlamydia* includes intracellular parasites, *C. trachomatis*, *C. psittaci* and *C. pneumoniae* [91]. Free living species are not known. Early studies showed that host DNA is not salvaged by the parasite [92,93]. Exogenous deoxyadenosine and deoxyguanosine are efficient DNA precursors in *C. psittaci*, but deoxycytidine and thymidine are poorly incorporated [91,94]. In a recent study, using a hypoxanthine–guanine phosphoribosyltransferase-negative cell line and inhibitors of salvage enzymes, it was shown that purine deoxyribonucleoside usage requires intact host catabolic pathways and salvage reactions [95]. The experiments exclude dependence of *Chlamydia* on host deoxynucleosides and provide indirect evidence for a chlamydial RNR.

Proteobacteria

The 'purple bacteria' include a wide array of phenotypes. Anaerobes and aerobes, heterotrophs and chemolithotrophs, photosynthetic and non-photosynthetic species are spread throughout the phylum.

α subdivision. Rhizobia (*R. meliloti*, *R. japonicum*, *R. leguminosarum* (formerly *R. trifolii* and *R. phaseoli*)) possess a B_{12}-dependent enzyme [96]. Cobalt deficiency induces the apoenzyme and stops DNA synthesis. Rescue of cobalt-limited cultures was not possible with deoxynucleosides indicating the absence of efficient salvage enzymes. *Agrobacterium tumefaciens* and *Rickettsia prowazekii* contain HU-sensitive RNRs [97,231]. *Paracoccus denitrificans* (formerly *Micrococcus denitrificans*) has a class II enzyme which is repressed under aerobic conditions [89]. The existence of a second RNR has not been investigated.

β subdivision. Our knowledge of deoxyribonucleotide formation in this subdivision is limited to growth inhibition of *Neisseria meningitidis* [98] and *Alcaligenes eutrophus* by hydroxyurea [99].

γ subdivision. Major members of this group are enterobacteria and pseudomonads. *E. coli* has two ribonucleotide reductases, a class I diphosphate reductase and a class IV triphosphate reductase. Due to the lack of studies on other aerobic RNRs it is not clear if the class I enzyme is of bacterial origin or has been acquainted in a horizontal gene transfer from a eukaryotic source. Growth of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis* is sensitive towards hydroxyurea [100–103]. The widespread occurrence of an aerobic enzyme in enterobacteria is also supported by negative tritium release assays with *Enterobacter aerogenes* (formerly *Aerobacter aerogenes*) and *Pseudomonas aeruginosa* [61,89]. However, ribonucleotide reduction in crude extracts of *Pseudomonas stutzeri* was performed in the presence of deoxyadenosylcobalamin [104]. The published data are not sufficient to determine the reductase class.

δ and ε subdivisions. Intensive research on anaerobic habitats during the last decade accounts for several isolates located in the δ and ε subgroups: sulphate and sulphur-reducing bacteria [105–107], *Metallobacter oxidoreducens* [108], *Wolinella succinogenes* [109] and *Pelobacter* sp. [110]. However, studies on DNA precursor biosynthesis have been limited to aerobes, predominantly in bdellovibrios. A myxobacter isolate reacted negative in the tritium release assay [89].

DNA replication of *Bdellovibrio* sp. occurs only in the host cell after invasion into the periplasmic space. Experiments with [^{3}H]thymidine labelled *E. coli* showed that host DNA is degraded by bdellovibrio deoxyribonucleases and incorporated into bdellovibrio DNA [111,112]. Exogenous thymidine monophosphates compete effectively with labelled host DNA; low incorporation rates of thymidine argue for a deficiency of deoxynucleoside kinases [113]. Interactions between host and bdellovibrio control the initiation of DNA precursor biosynthesis by host DNA degradation [114]. Bdellovibrio is certainly salvaging degraded host DNA for its DNA synthesis; however, it has not been investigated whether bdellovibrio can synthesize deoxyribonucleotides de novo.

Cyanobacteria

Cyanobacteria are the only prokaryotes performing oxygenic photosynthesis. The tritium exchange reaction has been used extensively to gain

insight into the taxonomic position of 'blue-green algae' [115,116]. Extracts from *Anabaena* 7119, *A. flos-aquae, Anacystis nidulans, Coccochloris peniocystis, Fremyella diplosiphon, Nostoc commune, Oscillatoria prolifera, Phormidium autumnale, P. luridum, Plectonema boryanum, Scytonema hofmanni, Synechococcus* sp. 6311, 6312, 7401, 6908 and ATCC 27146 reacted positively in the assay. The halophilic *Aphanothece halophytica* is not sensitive towards hydroxyurea [117]. Purification from *Anabaena* 7119 yielded a monomeric enzyme [118]. *Agmenellum quadruplicatum* and *Synechococcus* sp. 7003 ('*Coccochloris elabens*') have not a class II RNR, but the enzyme structures have not been elucidated [115,116,119]. The mechanism of deoxyribonucleotide formation in prochlorophytes is not known [120].

Gram-positive bacteria

Firmicutes with high G + C Content of DNA. The deepest branchings in the high G + C subdivision are defined by anaerobic species, the bifidobacteria and the propionibacteria. The latter use a class II RNR [121,122]. The B_{12}-dependent enzyme has two major disadvantages: biosynthesis of the cofactor involves several proteins and consumes more energy than the synthesis of other RNRs. More importantly, the cobalt requirement limits available habitats. The low cobalt content in the environment [123] causes a selective pressure for an alternative RNR. As propionibacteria that possess catalases are aerotolerant, they had the opportunity to develop an oxygen-dependent RNR. It has indeed been reported that some strains induce a hydroxyurea-sensitive RNR under cobalt-deficient growth [122,124]. Induction of the enzyme requires iron ions in the media. The nature of the second RNR has not been determined.

Actinomycetes are predominantly respiratory strict aerobic soil bacteria and have evolved more recently according to phylogenetic studies. The manganese RNR has been characterized from *Corynebacterium ammoniagenes* (formerly *Brevibacterium ammoniagenes*) and, as evident from growth studies, is also present in *Nocardia opaca, Arthrobacter globiformis, A. oxydans, A. citreus* and *Micrococcus luteus* (formerly *M. lysodeikti-*

cus) [9,27,52,125,126]. *Mycobacterium smegmatis* is sensitive towards hydroxyurea [127,128]. *Micrococcus luteus* does not contain a B_{12}-dependent RNR [89]. Actinomycetes in mammalian hosts are often able to grow anaerobically. *Corynebacterium nephridii* was isolated from urine of horse leech [129] and contains a B_{12}-dependent RNR [130]. Enzyme activity was also determined with the tritium release assay and increased almost 10-fold when *Corynebacterium* was grown anaerobically with nitrate as electron acceptor [89]. This anaerobic induction of the class II enzyme suggests the presence of a second aerobic RNR in *Corynebacterium nephridii. Streptomyces aureofaciens* also contains a cobalt-dependent enzyme [131].

Firmicutes with low G + C content of DNA. The evolution of RNRs in this subdivision of Gram-positive bacteria seems to follow the developmental path observed in the G + C-rich subdivision. Anaerobic bacteria representing deep lineages possess a cobalamin-dependent enzyme, whereas more recently evolved aerobic species contain a hydroxyurea-sensitive RNR. The lack of studies on the nature of these aerobic RNRs represents a major gap in our knowledge.

Clostridia (*C. sticklandii, C. tetanomorphum, C. thermoaceticum*) and lactobacilli (*L. leichmanii, L. casei, L. acidophilus*) possess a B_{12}-dependent RNR [25,61,89,132]. The latter have also evolved a functional salvage synthesis of deoxyribonucleotides [57,58]. *Lactobacillus acidophilus* R-26 contains, besides a thymidine kinase, two deoxynucleoside kinases specific for deoxycytidine/ deoxyadenosine and deoxyguanosine/ deoxyadenosine [133,134]. Studies with *Enterococcus faecalis* (formerly *Streptococcus faecalis*) indicate the presence of two RNRs. Aerobic growth is hydroxyurea-sensitive whereas anaerobic growth is not impaired by HU [135]. *Staphylococcus epidermis* is sensitive towards HU [136]. Mycoplasmas represent the fastest evolving family in this subdivision. These parasites seem to be HU-resistant but it has not been investigated if this is caused by salvage synthesis or a class II enzyme [137]. *Bacillus megaterium* has a class II RNR [138], whereas *Bacillus subtilis* possesses a HU-sensitive enzyme [100]. The presence of de-

oxyribonucleoside kinases in *B. subtilis* permits the isolation of RNR mutants [139,140]. The enzyme activity was found in a DNA-membrane multienzyme complex with replisome activity [141]. dATP salvage synthesis has been characterized in *B. cereus* [142].

Phylogenetically unaffiliated bacteria

Fusobacterium varius (formerly *Sphaerophorus varius*) is an obligate anaerobic Gram-negative rod-shaped bacteria and contains a deoxyadenosylcobalamin-dependent RNR [89].

Eucarya

Microsporidia, diplomonads and trichomonads

The deepest branch within the domain Eucarya is represented by microsporidia which are obligate intracellular parasites without mitochondria [143,144]. Deoxyribonucleotide formation was studied in their closest living relative, the diplomonad *Giardia lamblia* [145,146]. The anaerobic gut inhabitant lacks de novo synthesis of purines, pyrimidines and deoxyribonucleotides. DNA synthesis relies completely on salvage of deoxynucleosides. The lack of a RNR was also observed in *Trichomonas vaginalis* which contains deoxyribonucleoside phosphotransferases [147]. A RNR activity was found in *Tritrichomonas foetus* extracts, independent of B_{12} and insensitive to hydroxyurea [148]. To determine this, a nucleoside assay was used. Therefore it cannot be excluded from the available data that a false-positive salvage activity was observed.

Euglenids

The flagellate branchings euglenids and trypanosomes might stem from the microaerobic period in the earth's history, emerging before the major burst of eukaryotic lineages: ciliates, dinoflagellates, fungi, plants and animals [7]. *Euglena gracilis* is the descendant of the first photosynthetic *Eukaryon*. DNA synthesis depends on a class II RNR and it has been used as microbiological assay for B_{12} [149,150]. *Euglena* exhibits also a high sensitivity towards HU which inhibits chlorophyll biosynthesis [42]. This is the only known case where HU inhibition of growth at low

HU concentrations is not based on DNA precursor limitation. The presence of a cobalt-dependent RNR was also detected in a non-photosynthetic euglenid. Cell extracts of *Astasia longa* react positive in the tritium exchange/release assay [89].

Trypanosomes

A RNR from trypanosomes has not been described, but all members of the genus investigated so far are susceptible to hydroxyurea. Growth rates of *Trypanosoma cruzi*, *T. gambiense* and *Leishmania donovani* were halved just above 100 μM HU [145]. Other studies reported I_{50}-values up to 2 mM HU for *T. brucei* [151,152], *Crithidia fasciculata* [153] and *C. luciliae* [154]. HU inhibited cell proliferation of *L. tarentolae* [155], *L. major* [156], *L. donovani* [156], *L. mexicana* [157] and *Herpetomonas megaseliae* [158]. The stimulation of [^{3}H]thymidine uptake in the presence of low HU concentration observed in several cases is a typical physiological reaction caused by the lack of deoxyribonucleotides [152,159].

Replication of kinetoplast DNA seems to be HU-insensitive in several species. Cell cycle inhibition of *H. megaseliae* with HU lead to enrichment of multiflagellates with multiple kinetoplasts, indicating an evidently differential inhibitory effect of the drug on nuclear division versus kinetoplast DNA replication [158]. Enrichment of 2K-1N forms was observed with *T. brucei brucei* procyclic culture forms [152]. These results could not be confirmed in short-term inhibition experiments with *C. luciliae* [154], *L. tarentolae* [155] and *T. brucei* [160].

Slime moulds

The source of DNA precursors in *Entamoeba* is not known [161]. Cell proliferation of *Physarum polycephalum* and *Dictyostelium discoideum* is inhibited by hydroxyurea [162,163]. The nature of ribonucleotide reductases involved has not been studied.

Ciliates

Cell biological studies revealed that proliferation and DNA synthesis in *Tetrahymena pyri-*

formis and *Blespharisma seshachari* are sensitive towards hydroxyurea [164–167]. The effect is reversible and can be relieved with deoxyribonucleosides [168].

Free-living ciliates have evolved to exploit anaerobic habitats. Their organelles morphologically resemble mitochondria, but lack mitochondrial enzymes. The hydrosomes seem to be modified mitochondria and differ from the hydrosomes of trichomonads which may have evolved from an endosymbiontic fermenting bacterium [144]. Deoxyribonucleotide formation may be based on salvage supply, an anaerobic RNR or supply by endosymbiontic bacteria. Many anaerobic ciliates contain methanogenic bacteria as endosymbionts which support growth by uptake of hydrogen. Growth of anaerobic ciliates without endosymbionts has been achieved, therefore excluding an endosymbiontic DNA precursor supply [169,170].

Parasitic sporozoa: Plasmodium and Toxoplasma

Growth of *Plasmodium falciparum* and *Toxoplasma gondii* is inhibited by hydroxyurea [171–173]. Chemical mutagenesis was used to isolate a hydroxyurea-resistant mutant of *T. gondii* [173].

Algae, plants, fungi and animals

Tritium release assays performed on green algae were negative [89,116]. Specific growth inhibition by hydroxyurea was observed with *Acetabularia*, *Chlamydomonas* and *Charavulgaris* [174–177]. RNR activity could easily be detected in crude extracts of *Chlorella pyrenoidosa* and *Scenedesmus obliquus* [178]. The enzyme of *S. obliquus* was characterized and exhibits the typical EPR signal of a class I RNR [179–181]. The hydroxyurea-sensitivity of RNR activity in plant extracts supports the general impression that all higher eukaryotes have a class I enzyme [182,183].

Ribonucleotide reduction has been studied in great detail in *Saccharomyces cerevisiae*. Genes for the small subunit R2 and two large subunits R1 of a class I RNR were isolated [184–187]. The second large subunit is only expressed in response to DNA damage, in addition to the regular large subunit [188]. It is the first example of a special enzyme existing for DNA repair. Thioredoxin deficiency prolongs the S phase [189]. The active RNR exhibits the tyrosyl radical EPR signal of class I enzymes [190]. It has long been known that *S. cerevisiae* can grow, but not proliferate under anaerobic conditions [191,192]. An anaerobic RNR activity was not detectable [190].

Proliferation inhibition caused by hydroxyurea was observed in cultures of *Wangiella dermatitidis* [193], *Candida albicans* [194], *Neurospora crassa* [195,196] and *Schizophyllum commune* [197–199]. RNR activity in extracts of the water mould *Achyla* was also hydroxyurea-sensitive [200]. Only one short communication reported evidence for a cobalamin-dependent enzyme in fungi: the RNR activity of *Pithomyces chartarum* extracts required B_{12} [201].

The class I RNR from mammals differs slightly from the *E. coli* enzyme: the tyrosyl radical has a short half-life, iron ions are removed more easily from the protein and the reactivation of hydroxyurea-inhibited enzyme preparations is easily achieved in vitro. The instability of the active enzyme allows one to understand why mammalian cells exhibit an extended G_1/S transition under moderate limitations of oxygen tension [202–204].

Viruses

The genome of coliphage T4 includes genes for a class I (*nrdAB*) and a class IV (*sunY*) enzyme [29,205,206]. Class I enzymes were found in Teven and T5 coliphages using aerobic cultures and in large mammalian viruses. The protein environment of the tyrosyl radical is different in the R2 protein of herpes virus, mouse and *E. coli* [207]. Recently, a hydroxyurea-sensitive enzyme has been detected in phycovirus-infected green algae [232].

Ecology of ribonucleotide reduction

Chemical opportunities for radical domains

Evolution of life has led to a limited number of structures usable for radical enzymology. Every one-electron transfer reaction involves molecules

with unpaired electrons, either transition metal ions or organic radicals. Radicals are present in electron transport chains, they are intermediates in several oxygenation reactions [208–215]. But the number of reactions known to involve catalytic organic radicals is short. Activation of prostaglandin H synthase with peroxides yields a high-valency ferryl heme and a tyrosyl radical which catalyzes the cyclooxygenase reaction [216,217]. Several radical-induced rearrangements depend on deoxyadenosylcobalamin (Vitamin B_{12}) [215,218–220]. The coenzyme is substituted by S-adenosylmethionine, cobalt and iron–sulphur clusters in lysine 2,3-aminomutase [74–78] and by an organic free radical in a membrane-bound dioldehydratase in *Clostridium glycolicum* [221,222]. The highest numbers of possible radical domains for one reaction has been detected in RNRs. A glycine radical is the active species in pyruvate–formate lyase [72].

Catalysis via radicals requires tight control on possible reaction pathways of the highly reactive intermediates [215]. Proteins are ideal substrates for remodelling spontaneously formed structures for different purposes. The non-heme iron clusters present in class I RNR occur also in methane monooxygenase from methylotrophs, e.g. *Methylococcus capsulatus* and *Methylosinus trichosporium*, in hemerythrin, a reversible O_2 binding and transport protein in blood of marine invertebrates, in purple phosphatases and in ruberythrin from *Desulfovibrio* sp. [223,224]. Ruberythrin is a periplasmic protein with a midpoint redox potential of +230 mV and might serve as oxygen reductase in oxygen-tolerant sulphate-reducing bacteria. The diversity of enzymatic functions realized with one basic structure is impressive and has a complement in the number of structures adapted to fulfil one function: ribonucleotide reduction. Further studies will reveal which other structures have been adopted for radical domains.

Ribonucleotide reduction within cells

The importance of constant intracellular deoxyribonucleotide pools for replication fidelity explains the tight regulation of ribonucleotide re-

ductase activity during the cell cycle. Levels of regulation include gene expression, translational control, stability of the proteins, posttranslational activation and deactivation and allosteric control of enzyme activity by intracellular ATP and dNTP pool sizes. The nucleotide pools are influenced by salvage, catabolism and substrate cycles. Regulation occurs during the cell cycle and spatially within the cell. The intracellular organization of dNTP and DNA synthesis is discussed in the framework of a 'replitase': a supramolecular structure built through loose association of several enzymes involved in DNA precursor biosynthesis and DNA replication, channels the intermediates to receive high local concentrations of dNTPs at the origin of DNA replication. Although experimental data deny the existence of a 'replitase' in mammalian cells, a dNTP synthase complex has been characterized from T4-infected *E. coli* [225,226] and DNA replication occurs within a multienzyme complex. Most purified ribonucleotide reductase preparations have poor in vitro activities insufficient to sustain in vivo rates of DNA replication. One possible explanation could be the lack of activity stimulation by protein–protein interaction within a multienzyme complex. For prokaryotes, a 'replitase' is still an attractive model and is supported by experimental data, e.g. the purification of a replisome from *Bacillus subtilis* [141]. Further genetic and biochemical studies have to prove that protein–protein interaction in replitases are required for efficient dNTP synthesis.

Deoxyribonucleotides for replication of mitochondrial and chloroplast DNA are imported and not synthesized in the cell organelles. Mitochondria and chloroplasts – as known from mammals and plants – represent the evolutionary final stage of a symbiotic integration of a bacterium in a eukaryotic host cell where only a special part of the metabolism is maintained in the organelle for the benefit of the organism. The presence of a RNR seems possible in organelles which still have an elaborate metabolisms. Growth studies of trypanosomes in the presence of hydroxyurea occasionally revealed a mitochondrial DNA replication [152,158]. The biochemistry behind this observation deserves attention.

Ribonucleotide reduction and oxygen

The availability of molecular oxygen defines life spheres: anaerobic and aerobic habitats. Evolution of molecular oxygen was a major event in the earth's history that strongly influenced the evolution of life. The oxygen requiring RNRs (class I and III) are based on metal clusters available by spontaneous formation and represent enzymes developed after oxygen evolution. The class I enzyme has been purified from several eukaryotes which evolved after the oxygen burst. It is also present in *E. coli* – living in the digestive tracts of humans – and viruses infecting mammalian cells or *E. coli*. The presence of an ancient anaerobic RNR in *E. coli*, maintained by selective pressure in the anaerobic gut environment, raises questions about the heritage of the class I enzyme. A horizontal gene transfer is an attractive explanation, considering the ease of a gene transfer mechanism compared with the unlikelihood of accidental duplicate enzyme evolution. However, our knowledge of aerobic HU-sensitive RNRs in proteobacteria is very limited and does not allow any speculation about the direction of the gene transfer. The presence of two RNR in one organism seems to be widespread among microorganisms. A careful assessment of published data indicates that *Paracoccus denitrificans*, propionibacteria, *Corynebacterium nephredii* and *Enterococcus faecalis* are able to adapt deoxynucleoside synthesis to environmental limitations.

Phylogenetic trees based on ribosomal RNA sequences used as molecular chronometers indicate that major bacterial groups evolved before the rise in oxygen levels. Organisms using a class II enzyme are prepared to live in an aerobic environment, although the usage of a cobalt-dependent RNR restricts the habitat. Occupation of aerobic environments with cobalt-deficiency required innovation of a new RNR. The manganese enzyme is one archetype of a successful adaption. We do not know how archaebacteria adapted to aerobic environments, e.g. halobacteria or sulphidogens.

The anaerobic class II and IV enzymes use RNA-derived cofactors. Amino acids and nucleosides are easily formed under prebiotic conditions. Therefore, one might suggest that the *S*-adenosylmethionine-dependent glycine radical enzyme represents an older RNR than the B_{12}-dependent RNR, perchance the ancient enzyme. Eschenmoser's studies on abiotic formation of porphyrinogenic structures have shown that the chromophore is easily accessible starting from α-aminonitriles [233]. This observation, together with the widespread occurrence of class II enzymes, counterbalance the arguments favouring class IV enzymes. Finally, dNTP synthesis has not been investigated in several old bacteria.

Other anaerobic RNRs have not been characterized completely, but at present studies on deoxyribonucleotide formation in methanogens indicate that a class V enzyme occurs in archaebacteria. The currently pursued study on *Methanobacterium thermoautotrophicum* is the first in-depth study on archeal RNRs.

Deoxyribonucleotide formation in anaerobic eukaryotes has only been studied in organisms which represent ancient species and depend nowadays on salvage synthesis, e.g. *Giardia lamblia*. A second group of organisms consisting of protozoa and fungi have re-explored eukaryotic life in anaerobic habitats. Anaerobic fungi have been isolated from faeces and gut [227–230]. Free-living anaerobic ciliates contain endosymbiontic bacteria which live on hydrogen produced by the host. The source of deoxyribonucleotides in these anaerobic eukaryotes is unknown.

Outlook: Evolution-oriented research on ribonucleotide reduction

Studies on deoxyribonucleotide formation were initiated by interest in the basic metabolic pathways of living organisms and have been maintained by the complex structure of the enzyme and our difficulties to understand the enzyme mechanism. The central function of RNRs in DNA replication and cell proliferation initiated intensive basic research on eukaryotic and viral RNRs in order to develop drugs against cancer and viral diseases.

Renewed interest on RNR research results from studies on the evolution of life. Phylogenetic studies on ribosomal RNA provided the first view on the early evolutionary history of organisms. The new picture of biological evolution has started a hefty discussion on the nature of the common ancestor, the first DNA organism. Currently, our fractal knowledge of the nature and occurrence of ribonucleotide reductases cannot contribute to an understanding of the subject. Future studies on deoxyribonucleotide formation in evolutionary relevant organisms will support us in painting a picture of the RNA–DNA transition.

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References

1 Gilbert, W. (1986) The RNA world. Nature 319, 618.

2 Watson, J.D., Hopkins, N.H., Roberts, J.W., Steitz, J.A. and Weiner, A.M. (1987) Molecular biology of the gene, pp. 1140–1142. Benjamin/Cummings, Menlo Park, CA.

3 Follmann, H. (1982) Deoxyribonucleotide synthesis and the emergence of DNA in molecular evolution. Naturwissenschaften 69, 75–81.

4 Follmann, H. (1986) Have deoxyribonucleotides and DNA been among the earliest biomolecules? Adv. Space Res. 6, 33–38.

5 Shapiro, R. (1988) Prebiotic ribose synthesis, a critical analysis. Orig. Life Evol. Biosphere 18, 71–85.

6 Benner, S.A., Ellington, A.D. and Tauer, A. (1989) Modern metabolism as a palimpsest of the RNA world. Proc. Natl. Acad. Sci. USA 86, 7054–7058.

7 Woese, C.R. (1987) Bacterial evolution. Microbiol. Rev. 51, 221–271.

8 Olsen, G.J., Larsen, N. and Woese, C.R. (1991) The ribosomal RNA database project. Nucleic Acids Res. 19, Supplement, 2017–2021.

9 Lammers, M. and Follmann, H. (1983) The ribonucleotide reductases – a unique group of metalloenzymes essential for cell proliferation. Struct. Bonding (Berlin) 54, 27–91.

10 Ashley, G.W. and Stubbe, J. (1985) Current ideas on the chemical mechanism of ribonucleotide reductases. Pharmacol. Ther. 30, 301–329.

11 Stubbe, J. (1990) Ribonucleotide reductases. Adv. Enzymol. 63, 349–419.

12 Fontecave, M., Nordlund, P., Eklund, H. and Reichard, P. (1992) The redox centers of ribonucleotide reductase of Escherichia coli. Adv. Enzymol. 65, 147–183.

13 Reichard, P. (1987) Regulation of deoxyribotide synthesis. Biochemistry 26, 3245–3248.

14 Reichard, P. (1988) Interactions between deoxyribonucleotide and DNA synthesis. Ann. Rev. Biochem. 57, 349–374.

15 Meuth, M. (1989) The molecular basis of mutations induced by deoxyribonucleoside triphosphate pool imbalances in mammalian cells. Exp. Cell Res. 181, 305–316.

16 Holmgren, A. (1989) Thioredoxin and glutaredoxin systems. J. Biol. Chem. 24, 13963–13966.

17 Russel, M., Model, P. and Holmgren, A. (1990) Thioredoxin or glutaredoxin in Escherichia coli is essential for sulfate reduction but not for deoxyribonucleotide synthesis. J. Bacteriol. 172, 1923–1929.

18 Mao, S.S., Johnston, M.I., Bollinger, J.M. and Stubbe, J. (1989) Mechanism-based inhibition of a mutant Escherichia coli ribonucleotide reductase (cysteine-225 to serine) by its substrate CDP. Proc. Natl. Acad. Sci. USA 86, 1485–1489.

19 Åberg, A., Hahne, S., Karlsson, M., Larsson, A., Ormö, M., Åhgren, A. and Sjöberg, B.-M. (1989) Evidence for two different classes of redox-active cysteines in ribonucleotide reductase of Escherichia coli. J. Biol. Chem. 264, 12249–12252.

20 Karlsson, M., Sahlin, M. and Sjöberg, B.-M. (1992) Escherichia coli ribonucleotide reductase – radical susceptibility to hydroxyurea is dependent on the regulatory state of the enzyme. J. Biol. Chem. 267, 12622–12626.

21 Mao, S.S., Yu, G.X., Chalfoun, D. and Stubbe, J. (1992) Characterization of C439SR, a mutant of Escherichia coli ribonucleotide diphosphate reductase: evidence that C439 is a residue essential for nucleotide reduction and C439SR1 is a protein possessing novel thioredoxin-like activity. Biochemistry 31, 9752–9759.

22 Mao, S.S., Holler, T.P., Yu, G.X., Bollinger, J.M., Booker, S., Johnston, M.I. and Stubbe, J. (1992) A model for the role of multiple cysteine residues involved in ribonucleotide reduction: amazing and still confusing. Biochemistry 31, 9733–9743.

23 Mao, S.S., Holler, T.P., Bollinger, J.M., Jr., Yu, G.X., Johnston, M.I. and Stubbe, J. (1992) Interaction of C225SR1 mutant subunit of ribonucleotide reductase with R2 and nucleoside diphosphates: tales of a suicidal enzyme. Biochemistry 31, 9744–9751.

24 Elgren, T.E., Lynch, J.B., Juarez-Garcia, C., Münck, E., Sjöberg, B.-M. and Que, L., Jr. (1991) Electron transfer associated with oxygen activation in the B2 protein of ribonucleotide reductase from Escherichia coli. J. Biol. Chem. 266, 19265–19268.

25 Blakley, R.L. (1982) Cobalamin-dependent ribonucleotide reductases. In: B_{12} volume 2 (Dolphin, D., Ed.), pp. 381–418. John Wiley, New York, NY.

26 Hogenkamp, H.P.C. (1984) Nature and properties of the bacterial ribonucleotide reductases. Pharmacol. Ther. 23, 393–405.

27 Willing, A., Follmann, H. and Auling, G. (1988) Ribonucleotide reductase of Brevibacterium ammoniagenes is a manganese enzyme. Eur. J. Biochem. 170, 603–611.

28 Harder, J., Eliasson, R., Pontis, E., Ballinger, M.D. and Reichard, P. (1992) Activation of the anaerobic ribonucleotide reductase from Escherichia coli by S-adenosylmethionine. J. Biol. Chem. 267, 25548–25552.

29 Sun, X., Harder, J., Krook, M., Jörnvall, H., Sjöberg, B.-M. and Reichard, P. (1993) A possible glycine radical in anaerobic ribonucleotide reductase from Escherichia coli. Nucleotide sequence of the cloned nrdD gene. Proc. Natl. Acad. Sci. USA 2, 577–581.

30 Mulliez, E., Fontecave, M., Gaillard, J. and Reichard, P. (1993) An iron-sulfur center and a free radical in the active anaerobic ribonucleotide reductase of Escherichia coli. J. Biol. Chem. 268, 2296–2299.

31 Steeper, J.R. and Steuart, C.D. (1970) A rapid assay for CDP reductase activity in mammalian cell extracts. Anal. Biochem. 34, 123–130.

32 Harder, J., Follmann, H. and Hantke, K. (1989) Deoxyribonucleotide synthesis in an Escherichia coli mutant (H 1491) which lacks ribonucleotide reductase subunit B2. Z. Naturforsch. 44c, 715–718.

33 Steenkamp, D.J. (1991) The purine-2-deoxyribonucleosidase from Crithidia luculiae. Eur. J. Biochem. 197, 431–439.

34 Reichard, P., Baldesten, A. and Rutberg, L. (1961) Formation of deoxycytidine phosphates from cytidine in extracts from Escherichia coli. J. Biol. Chem. 236, 1150–1157.

35 Yeh, Y.-C. (1978) A simple and sensitive assay procedure for ribonucleotide reductase system. Anal. Biochem. 86, 175–183.

36 Sato, K., Seki, T., Inukai, S. and Shimizu, S. (1981) A simple and sensitive method for assay of a ribonucleotide reductase system. Anal. Biochem. 116, 185–188.

37 Narine, D.R., Bacchetti, S. and Chan, W.W.-C. (1985) An assay for ribonucleotide reductase based on ion-exchange chromatography of the reaction product. Anal. Biochem. 145, 331–338.

38 Petersson, L., Gräslund, A., Ehrenberg, A., Sjöberg, B.-M. and Reichard, P. (1980) The iron center in ribonucleotide reductase from Escherichia coli. J. Biol. Chem. 255, 6706–6712.

39 Bollinger, J.M., Jr., Edmondson, D.E., Huynh, B.H., Filley, J., Norton, J.R. and Stubbe, J. (1991) Mechanism of assembly of the tyrosyl radical-dinuclear iron cluster cofactor of ribonucleotide reductase. Science 253, 292–298.

40 Turner, M.D., Abrams, R. and Lieberman, I. (1966) Mesodiphenylsuccinate and hydroxyurea as inhibitors of deoxycytidylate synthesis in extracts of Ehrlich ascites and L-cells. J. Biol. Chem. 241, 5777–5780.

41 Moore, E.C. and Hurlbert, R.B. (1985) The inhibition of ribonucleotide diphosphate reductase by hydroxyurea, guanazole and pyrzoloimidazole (IMPY). Pharmacol. Ther. 27, 167–196.

42 Buetow, D.E. and Mego, J.L. (1967) Hydroxyurea inhibition of greening, macromolecule synthesis and cell division in Euglena gracilis. Biochim. Biophys. Acta 134, 395–401.

43 Reichard, P. (1989) Commentary on 'Formation of deoxycytidine 5'-phosphate from cytidine 5'-phosphate with enzymes from Escherichia coli' by P.Reichard and L. Rutberg, Biochim. Biophys. Acta 37 (1960) 554–555. Biochim. Biophys. Acta 1000, 49–50.

44 Nordlund, P., Sjöberg, B.-M. and Eklund, H. (1990) Three-dimensional structure of the free radical protein of ribonucleotide reductase. Nature 345, 593–598.

45 Reichard, P. and Ehrenberg, A. (1983) Ribonucleotide reductase – a radical enzyme. Science 221, 514–519.

46 Gräslund, A., Sahlin, M. and Sjöberg, B.-M. (1985) The tyrosyl free radical in ribonucleotide reductase. Environ. Health Perspect. 64, 139–149.

47 Eriksson, S. and Sjöberg, B.-M. (1989) Ribonucleotide reductases. In: Allosteric Enzymes (Hervè, G., Ed.) pp. 189–215, CRC Press, Boca Raton, FL.

48 Bollinger, J.M., Jr., Stubbe, J., Huynh, B.H. and Edmondson, D.E. (1991) Novel diferric radical intermediate responsible for tyrosyl radical formation in assembly of the cofactor of ribonucleotide reductase. J. Am. Chem. Soc. 113, 6289–6291.

49 Climent, I., Sjöberg, B.-M. and Huang, C.Y. (1992) Site-directed mutagenesis and deletion of the carboxyl terminus of Escherichia coli ribonucleotide reductase protein R2. Effects on catalytic activity and subunit interaction. Biochemistry 31, 4801–4807.

50 Climent, I., Sjöberg, B.-M. and Huang, C.Y. (1991) Carboxyl-terminal peptides as probes for Escherichia coli ribonucleotide reductase subunit interaction: kinetic analysis of inhibition studies. Biochemistry 30, 5164–5171.

51 Nordlund, P. (1990) Structural studies on the free radical protein of ribonucleotide reductase. PhD thesis, Uppsala, Sweden.

52 Auling, G., Thaler, M. and Diekmann, H. (1980) Parameters of unbalanced growth and reversible inhibition of deoxyribonucleic acid synthesis in Brevibacterium ammoniagenes ATCC 6872 induced by depletion of Mn^{2+}. Inhibitor studies on the reversibility of deoxyribonucleic acid synthesis. Arch. Microbiol. 127, 105–114.

53 Schimpff-Weiland, G., Follmann, H. and Auling, G. (1981) A new manganese-activated ribonucleotide reductase found in gram-positive bacteria. Biochem. Biophys. Res. Comm. 102, 1276–1282.

54 Willing, A., Follmann, H. and Auling, G. (1988) Nucleotide and thioredoxin specificity of the manganese ribonucleotide reductase from Brevibacterium ammoniagenes. Eur. J. Biochem. 175, 167–173.

55 Brudvig, G.W., Beck, W.F. and de Paula, J.C. (1989) Mechanism of photosynthetic water oxidation. Ann. Rev. Biophys. Biophys. Chem. 18, 25–46.

56 Wieghardt, K. (1989) The active sites in manganese-containing metalloproteins and inorganic model complexes. Angew. Chem. Int. Ed. Engl. 28, 1153–1172.

57 Kitay, E., McNutt, W.S. and Snell, E.E. (1950) Deoxyribosides and vitamin B_{12} as growth factors for lactic acid bacteria. J. Bacteriol. 59, 727–738.

58 Downing, M. and Schweigert, D.S. (1956) Role of vitamin B_{12} in nucleic acid metabolism. IV. Metabolism of ^{14}C-labeled thymidine by Lactobacillus leichmannii. J. Biol. Chem. 220, 521–526.

59 Blakley, R.L. and Barker, H.A. (1964) Cobamide stimulation of the reduction of ribotides to deoxyribotides in Lactobacillus leichmannii. Biochem. Biophys. Res. Comm. 16, 391–397.

60 Gleason, F.K. and Hogenkamp, H.P.C. (1971) Preparation of 5'-deoxyadenosylcobalamin-5'-$^3H_2$. Methods Enzymol. 18c, 65–71.

61 Abeles, R.H. and Beck, W.S. (1967) The mechanism of action of cobamide coenzyme in the ribonucleotide reductase reaction. J. Biol. Chem. 242, 3589–3593.

62 Hogenkamp, H.P.C., Ghambeer, R.K., Brownson, C., Blakley, R.L. and Vitols, E. (1968) Cobamides and ribonucleotide reduction. J. Biol. Chem. 243, 799–808.

63 Ashley, G.W., Harris, G. and Stubbe, J. (1986) The mechanism of Lactobacillus leichmanii ribonucleotide reductase. J. Biol. Chem. 261, 3958–3964.

64 Lin, A.-N.I., Ashley, G.W. and Stubbe, J. (1987) Location of the redox-active thiols of ribonucleotide reductase: sequence similarity between the Escherichia coli and Lactobacillus leichmannii enzymes. Biochemistry 26, 6905–6909.

65 Ashley, G.W. Harris, G. and Stubbe, J. (1988) Inactivation of the Lactobacillus leichmannii ribonucleoside triphosphate reductase by 2'-chloro-2'deoxyuridine 5'-triphosphate: stoichiometry of inactivation, site of inactivation, and mechanism of the protein chromophore formation. Biochemistry 27, 4305–4310.

66 Ashley, G.W., Harris, G. and Stubbe, J. (1988) Inactivation of the ribonucleoside triphosphate reductase from Lactobacillus leichmannii by 2'-chloro-2'-deoxyuridine 5'-triphosphate: a 3'-2' hydrogen transfer during the formation of 3'-keto-2'-deoxyuridine 5'-triphosphate. Biochemistry 27, 7841–7845.

67 Hantke, K. (1988) Characterization of an iron sensitive Mud 1 mutant in E. coli lacking the ribonucleotide reductase subunit B2. Arch. Microbiol. 149, 344–349.

68 Fontecave, M., Eliasson, R. and Reichard, P. (1989) Oxygen-sensitive ribonucleoside triphosphate reductase is present in anaerobic Escherichia coli. Proc. Natl. Acad. Sci. USA 86, 2147–2151.

69 Eliasson, R., Fontecave, M., Jörnvall, H., Krook, M., Pontis, E. and Reichard, P. (1990) The anaerobic ribonucleoside triphosphate reductase from Escherichia coli requires S-adenosylmethionine as a cofactor. Proc. Natl. Acad. Sci. USA 87, 3314–3318.

70 Pontis, E., Sun, X., Jörnvall, H., Krook, M. and Reichard, P. (1991) ClpB proteins copurify with the anaerobic Escherichia coli reductase. Biochem. Biophys. Res. Comm. 180, 1222–1226.

71 Eliasson, R., Pontis, E., Fontecave, M., Gerez, C., Harder, J., Jörnvall, H., Krook, M. and Reichard, P. (1992) Characterization of components of the anaerobic ribonucleotide reductase system from Escherichia coli. J. Biol. Chem. 267, 25541–25547.

72 Wagner, A.F.V., Frey, M., Neugebauer, F.A., Schäfer, W. and Knappe, J. (1992) The free radical in pyruvate formate-lyase is located on glycine-734. Proc. Natl. Acad. Sci. USA 89, 996–1000.

73 Zeeh, A. and Shub, D.A. (1991) The product of the split sunY gene of bacteriophage T4 is a processed protein. J. Bacteriol. 173, 6980–6985.

74 Moss, M. and Frey, P.A. (1987) The role of S-adenosylmethionine in the lysine 2,3-aminomutase reaction. J. Biol. Chem. 262, 14859–14862.

75 Moss, M. and Frey, P.A. (1990) Activation of lysine 2,3-aminomutase by S-adenosylmethionine. J. Biol. Chem. 265, 18112–18115.

76 Ballinger, M.D., Reed, G.H. and Frey, P.A. (1992) An organic radical in the lysine 2,3-aminomutase reaction. Biochemistry 31,949–953.

77 Petrovich, R.M., Ruzicka, F.J., Reed, G.H. and Frey, P.A. (1992) Characterization of iron-sulfur clusters in lysine 2,3-aminomutase by electron paramagnetic resonance spectroscopy. Biochemistry 31, 10774–10781.

78 Ballinger, M.D., Frey, P.A. and Reed, G.H. (1992) Structure of a substrate radical intermediate in the reaction of lysine 2,3-aminomutase. Biochemistry 31.10782–10789.

79 Hogenkamp, H.P.C., Follmann, H. and Thauer, R.K. (1987) Ribonucleotide reductase in cell extracts of Methanobacterium thermoautotrophicum. FEBS Lett. 219, 197–201.

80 Sze, I.S.-Y., McFarlan, S.C., Spormann, A., Hogenkamp, H.P.C. and Follmann, H. (1992) A possible new class of ribonucleotide reductase from Methanobacterium thermoautotrophicum. Biochem. Biophys. Res. Comm. 184, 1101–1107.

81 Lake, J.A. (1988) Origin of the eukaryotic nucleus determined by rate-invariant analysis of rRNA sequences. Nature 331, 184–186.

82 Cavalier-Smith, T. (1989) Archaebacteria and archezoa. Nature 339, 100–101.

83 Woese, C.R., Kandler, O. and Wheelis, M.L. (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria and Eucarya. Proc. Natl. Acad. Sci. USA 87, 4576–4579.

84 Lake, J.A. (1991) Tracing origins with molecular sequences: metazoan and eukaryotic beginnings. TIBS 16, 46–50.

85 Zillig, W. (1991) Comparative biochemistry of Archea and Bacteria. Curr. Opinion Gen. Develop. 1, 544–551.

86 Rivera, M.C. and Lake, J.A. (1992) Evidence that eukaryotes and eocyte prokaryotes are immediate relatives. Science 257, 74–76.

87 Oren, A. (1991) Anaerobic growth of halophilic

archeobacteria by reduction of fumarate. J. Gen. Microbiol. 137, 1387–1390.

88 Oren, A. and Trüper, H.G. (1990) Anaerobic growth of halophilic archeobacteria by reduction of dimethylsulfide and trimethylamine N-oxide. FEMS Microbiol. Lett. 35, 55–58.

89 Gleason, F.K. and Hogenkamp, H.P.C. (1972) 5′-deoxyadenosylcobalamin-dependent ribonucleotide reductase: a survey of its distribution. Biochim. Biophys. Acta 277, 466–470.

90 Sando, G.N. and Hogenkamp, H.P.C. (1973) Ribonucleotide reductase from Thermus X-1, a thermophilic organism. Biochemistry 12, 3316–3322.

91 Moulder, J.W. (1991) Interaction of chlamydiae and host cells in vitro. Microbiol. Rev. 55, 143–190.

92 Crocker, T.T., Pelc, S.R., Nielson, B.I., Eastwood, J.M. and Banks, J. (1962) Population dynamics and deoxyribonucleic acid synthesis in HeLa cells infected with an ornithosis agent. J. Infect. Dis. 115, 105–122.

93 Lin, H.S. (1968) Stability of the nucleic acids of L cells after infection with the meningopneumonitis agent. J. Bacteriol. 96, 2054–2065.

94 Tribby, I.I.E. and Moulder, J.W. (1966) Availability of bases and nucleosides as precursors of nucleic acids in L cells and in the agent of meningopneumonitis. J. Bacteriol. 91, 2362–2367.

95 McClarty, G. and Tipples, G. (1991) In situ studies on incorporation of nucleic acid precursors into Chlamydia trachomatis DNA.J. Bacteriol. 173, 4922–4931.

96 Cowles, J.R., Evans, H.J. and Russell, S.A. (1969) B$_{12}$ coenzyme-dependent ribonucleotide reductase in Rhizobium species and the effects of cobalt deficiency on the activity of the enzyme. J. Bacteriol. 97, 1460–1465.

97 Kowalczyk, J. (1983) Effect of N-hydroxyurea, mitomycin C and actinomycin D on tumor formation on the leaves of Kalanchoe daigremontiana. Acta Soc. Bot. Pol. 52, 61–69.

98 Jyssum, K. (1973) Cell division after inhibition of DNA and protein synthesis in Neisseria meningitidis. Acta Pathol. Microbiol. Scand. Section B 81, 127–137.

99 Im, D.-S. and Friedrich, C.G. (1984) N-hydroxides, aminotriazole and cyamide activate ribulosebisphosphate carboxylase formation in Alcaligenes eutrophus. FEMS Microbiol. Lett. 25, 143–147.

100 Filpula, D. and Fuchs, J.A. (1979) Increased synthesis of ribonucleotide reductase after deoxyribonucleic acid inhibition in various species of bacteria. J. Bacteriol. 139, 694–696.

101 Gale, G.R., Kendall, S.M., McLain, H.H. and DuBois, S. (1964) Effect of hydroxyurea on Pseudomonas aeruginosa. Cancer Res. 24, 1012–1019.

102 Hamilton-Miller, J.M.T. (1984) Antimicrobial activity of 21 anti-neoplastic agents. Br. J. Cancer 49, 367–369.

103 Perrone, G., Ferrari, C. and Arghittu, M. (1983) Inhibitors of bacterial urease: microbiological considerations on hydroxyurea. Boll. Ist. Sieroter. Milan. 61, 513–515.

104 Holt, R.G., Mehrotra, B.D. and Hamilton, F.D. (1985) Sedimentation analysis of ribonucleotide reductase activity in extracts of Pseudomonas stutzeri. Biochem. Biophys. Res. Comm: 128, 1044–1050.

105 Widdel, F. and Bak, F. (1992) Gram-negative mesophilic sulfate-reducing bacteria. In: The Prokaryotes, Second Edition (Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H., Eds.) pp. 3352–3378. Springer, Heidelberg, Germany.

106 Widdel, F. and Pfennig, N. (1992) The genus Desulfuromonas and other gram-negative sulfur-reducing eubacteria. In: The Prokaryotes, Second Edition (Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H., Eds.) pp. 3379–3389. Springer, Heidelberg, Germany.

107 Widdel, F. (1992) The genus Thermodesulfobacterium. In: The Prokaryotes, Second Edition (Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H., Eds.) pp. 3390–3392. Springer, Heidelberg, Germany.

108 Lovley, D.L., Giovannoni, S.J., White, D.C., Champine, J.E., Phillips, E.J.P., Gorby, Y.A. and Godwin, S. (1993) Geobacter metallireducens gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. Arch. Microbiol. 159, 336–344.

109 Tanner, A. and Paster, B.J. (1992) The genus Wolinella. In: The Prokaryotes, Second Edition (Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H., Eds.) pp. 3512–3523. Springer, Heidelberg, Germany.

110 Schink, B. (1992) The genus Pelobacter. In: The Prokaryotes, Second Edition (Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H., Eds.) pp. 3393–3399. Springer, Heidelberg, Germany.

111 Matin, A. and Rittenberg, A.C. (1972) Kinetics of deoxyribonucleic acid destruction and synthesis during growth of Bdellovibrio bacteriovorus strain 109D on Pseudomonas putida and Escherichia coli. J. Bacteriol. 111, 664.–673.

112 Rosson, R.A. and Rittenberg, S.C. (1979) Regulated breakdown of Escherichia coli deoxyribonucleic acid during intraperiplasmic growth of Bdellovibrio bacteriovorus 109J. J. Bacteriol. 140, 620–633.

113 Rittenberg, S.C. and Langley, D. (1975) Utilisation of nucleoside monophosphates per se for intraperiplasmic growth of Bdellovibrio bacteriovorus. J. Bacteriol. 121, 1137–1144.

114 Thomashow, M.F. and Cotter, T.W. (1992) Bdellovibrio host dependence: the search for signal molecules and genes that regulate the intraperiplasmic growth cycle. J. Bacteriol. 174, 5767–5771.

115 Gleason, F.K. (1978) Distribution of ribonucleotide reductase in the genus Synechococcus. FEMS Microbiol. Lett. 3, 241–243.

116 Gleason, F.K. and Wood, J.M. (1976) Ribonucleotide reductase in blue-green algae: dependence on adenosylcobalamin. Science 192, 1343–1344.

117 Yopp, J.H., Albright, G. and Miller, D.M. (1979) Effects

of antibiotics and ultraviolet radiation on the halophilic blue-green alga *Aphanothece halophytica*. Botanica Marina 12, 167–272.

118 Gleason, F.K. and Frick, T.D. (1980) Adenosylcobalamin-dependent ribonucleotide reductase from the blue-green algae, *Anabaena* sp. J. Biol. Chem. 255, 7728–7733.

119 Gleason, F.K. and Wood, J.M. (1976) Ribonucleotide reductase activity in ether-treated cells of *Agmenellum quadruplicatum*. J. Bacteriol. 128, 673–676.

120 Palenik, B. and Haselkorn, R. (1992) Multiple evolutionary origins of prochlorophytes, the chlorophyll b-containing prokaryotes. Nature 355, 265–270.

121 Iordan, E.P., Petukhova, N.I. and Vorob'eva, L.I. (1986) The regulatory action of vitamin B_{12} on the ribonucleotide reductase system from *Propionibacterium shermanii*. Mikrobiologiya 55, 553–558.

122 Iordan, E.P. and Petukhova, N.I. (1989) Reorganisation of the ribonucleotide reductase system in propionic bacteria with inhibited synthesis of vitamin B_{12}. Mikrobiologiya 58, 533–538.

123 Merian, E. (1991) Metals and their compounds in the environment: occurrence, analysis and biological relevance, pp. 879–894. VCH, Weinheim, D.

124 Iordan, E.P. (1992) Modulation of DNA synthesis in *Propionibacterium freudenreichii* subsp. *shermanii* under conditions of corrinoid limitation. Microbiol. 61, 229–233.

125 Plönzig, J. and Auling, G. (1987) Manganese deficiency impairs ribonucleotide reductase but not DNA replication in *Arthrobacter* species. Arch. Microbiol. 146, 396–401.

126 Auling, G. (1983) The effect of manganese limitation on DNA precursor biosynthesis during nucleotide fermentation with *Brevibacterium ammoniagenes* and *Micrococcus luteus*. Eur. J. Appl. Microbiol. Biotechnol. 18, 229–235.

127 Winder, F.G. and Barber, D.S. (1973) Effects of hydroxyurea, nalidixic acid and zinc limitation on DNA polymerase and ATP-dependent deoxyribonuclease activities of *Mycobacterium smegmatis*. J. Gen. Microbiol. 76, 189–196.

128 MacNaughton, A.W. and Winder, F.G. (1977) Increased DNA polymerase and ATP-dependent deoxyribonuclease activities following DNA damage in *Mycobacterium smegmatis*. Mol. Gen. Genet. 150, 301–308.

129 Büsing, K.H., Döll, W. and Freytag, K. (1953) Die Bakterienflora der medizinischen Blutegel. Arch. Mikrobiol. 19, 52–86.

130 Tsai, P.K. and Hogenkamp, H.P.C. (1980) The purification and characterization of an adenosylcobalamin-dependent ribonucleoside diphosphate reductase from *Corynebacterium nephridii*. J. Biol. Chem. 255, 1273–1278.

131 Kollárová, M., Halick'y, P., Bukovská, G. and Zelinka, J. (1983) Properties of ribonucleotide reductase from *Streptomyces aureofaciens*. Biologia (Bratislava) 38, 1189–1195.

132 Blakley, R.L., Ghambeer, R.K., Nixon, P.P. and Vitols, E. (1965) The cobamide-dependent ribonucleoside

triphosphate reductase of lactobacilli. Biochem. Biophys. Res. Comm. 20, 439–445.

133 Chakravarty, R., Ikeda, S. and Ives, D.H. (1984) Distinct sites for deoxyguanosine and deoxyadenosine phosphorylation on a monomeric kinase from *Lactobacillus acidophilus*. Biochemistry 23, 6235–6240.

134 Ikeda, S. and Ives, D.H. (1985) Multisubstrate analogs for deoxynucleoside kinases. J. Biol. Chem. 260, 12659–12664.

135 Lahti, R. and Heinonen, J. (1979) Aeration sensitizes *Streptococcus faecalis* to hydroxyurea. Folia Microbiol. 24, 445–448.

136 Feiner, R.R., Rosenkranz, H.S. and Coward, J.E. (1972) Effect of hydroxyurea on *Staphylococcus epidermidis*. Temporary loss of viability. Antimicrob. Agents Chemother. 2, 147–155.

137 Grüneisen, A., Rajewsky, M.F., Remmer, I. and Uschkoreit, J. (1975) Inhibition of [³H]thymidine incorporation by hydroxyurea: atypical response of mycoplasma-infected cells in culture. Exp. Cell Res. 90, 365–373.

138 Yau, S. and Wachsman, T. (1973) The *Bacillus megaterium* ribonucleotide reductase: evidence for a B_{12} coenzyme requirement. Mol. Cell. Biochem. 1, 101–105.

139 Rima, B.K. and Takahashi, I. (1978) Deoxyribonucleoside-requiring mutants of *Bacillus subtilis*. J. Gen. Microbiol. 107, 139–145.

140 Shivakumar, A.G. and Dubnau, D. (1978) Differential effect of hydroxyurea on the replication of plasmid and chromosomal DNA in *Bacillus subtilis*. J. Bacteriol. 136, 1205–1207.

141 Laffan, J.J., Skolnik, I.L., Hadley, D.A., Bouyea, M. and Firshein, W. (1990) Characterization of a multienzyme complex derived from a *Bacillus subtilis* DNA-membrane extract that synthesizes RNA and DNA precursors. J. Bacteriol. 172, 5724–5731.

142 Ipata, P.L., Gini, S. and Tozzi, M.G. (1985) In vitro 5-phosphoribosyl 1-pyrophosphate-independent salvage biosynthesis of ribo- and deoxyriboadenine nucleotides in *Bacillus cereus*. Biochim. Biophys. Acta 842, 84–89.

143 Cavalier-Smith, T. (1987) Eukaryotes with no mitochondria. Nature 326, 332–333.

144 Müller, M. (1988) Energy metabolism of protozoa without mitochondria. Ann. Rev. Microbiol. 42, 465–488.

145 Baum, K.F., Berens, R.L., Marr, J., Harrington, J.A. and Spector, T. (1989) Purine deoxynucleoside salvage in *Giardia lamblia*. J. Biol. Chem. 264, 21087–21090.

146 Adam, R.D. (1991) The biology of *Giardia* spp. Microbiol. Rev. 55, 706–732.

147 Wang, C.C. and Cheng, H.-W. (1985) The deoxyribonucleoside phosphotransferase of *Trichomonas vaginalis*. J. Exp. Med. 160, 987–1000.

148 Wang, C.C. (1984) Purine and pyrimidine metabolism in *Trichomonadidae* and *Giardia*: potential targets for chemotherapy. In: Biochemical Basis of Drug Action (Singer, T.P., Mansour, T., Ondarza, R.N., eds.) pp. 133–145. Academic Press, New York, NY.

149 Ross, G.I.M. (1952) Vitamin B_{12} assay in body fluids using *Euglena gracilis*. J. Clin. Pathol. 5, 250–256.

150 Hamilton F.D. (1974) Ribonucleotide reductase from *Euglena gracilis*. A 5-deoxyadenosylcobalamin-dependent enzyme. J. Biol. Chem. 249, 4428–4434.

151 Fricker, S.P. and Klein, R.A. (1983) Characterisation of DNA and protein synthesis in *Trypanosoma brucei* by using inhibitors. Biochem. Soc. Trans. 11, 366–367.

152 Brun, R. (1980) Hydroxyurea: effect on growth, structure, and [^{3}H]thymidine uptake of *Trypanosoma brucei* procyclic culture forms. J. Protozool. 27, 122–128.

153 Cosgrove, W.B., Skeen, M.J. and Hadjuk, S.L. (1979) Effects of hydroxyurea on *Crithidia fasciculata*. J. Protozool. 26, 643–648.

154 Steinert, M. (1969) Reversible inhibition of the division of *Chrithidia luciliae* by hydroxyurea and its use for obtaining synchronized cultures. FEBS Lett. 5, 291–294.

155 Simpson, L. and Braly, P. (1970) Synchronization of *Leishmania tarentolae* by hydroxyurea. J. Protozool. 17, 511–517.

156 North, T.W. and Wyler, D.J. (1987) DNA synthesis in promastigotes of *L. leishmania major* and *L. donovani*. Mol. Biochem. Parasitol. 22, 215–221

157 Hart, D.T., Vickerman, K. and Coombs, G.H. (1981) Transformation in vitro of *Leishmania mexicana* amastigotes to promastigotes: nutritional requirements and the effect of drugs. Parasitol. 83, 529–541.

158 Knight, S.A. (1976) Differentiation of *Herpetomonas megaseliae*: effects of hydroxyurea on morphology and growth. J. Parasitol. 62, 515–522.

159 Injeyan, H.S., Meerovitch, E. and McLaughlin, J. (1974) Anomalous enhancing effects of hydroxyurea on DNA synthesis. Life Sci. 14, 1687–1693.

160 Astolfi Filho, S., Pereira de Almeida, E.R. and Gander, E.S. (1978) The influence of hydroxyurea and colchicine on growth and morphology of *Trypanosoma cruzi*. Acta Trop. 35, 229–237.

161 McLaughlin, J. and Aley, S. (1985) The biochemistry and functional morphology of *Entamoeba*. J. Protozool. 32, 221–240.

162 Kelly, P.M., Schofield, P.N. and Walker, I.O. (1983) Histone gene expression in *Physarum polycephalum* 2. FEBS Lett. 161, 79–83.

163 Deering, R.A. and Michrina, C.A. (1982) Inhibitors of DNA precursor metabolism in *Dictyostelium discoideum*. Antimicrob. Agents Chemother. 21, 764–769.

164 Byfield, J.E. and Scherbaum, O.H. (1967) Incomplete inhibition of synchronized cell division by hydroxyurea and its relevance to the normal cellular life cycle. Nature 216, 1017–1018.

165 Worthington, D.H., Nachtwey, D.S. and Wagenaar, E.B. (1975) Hydroxyurea: morphological effects on the macronucleus of *Tetrahymena*. Exp. Cell Res. 92, 435–443.

166 Klenow, S. (1982) Inhibition of DNA synthesis does not influence the H1 histone synthesis in *Tetrahymena pyriformis*. Biochim. Biophys. Acta 699, 49–52.

167 Sundararaman, V. and Dass, C.M.S. (1971) Effect of hydroxyurea on the development of macronuclear anlagen in the exconjugants of *Blespharisma seshachari*. Indian J. Exp. Biol. 9, 345–349.

168 Cameron, I.L. and Jeter, J.R. Jr. (1973) Action of hydroxyurea and *N*-carbamoyloxyurea on the cell cycle of *Tetrahymena*. Cell Tissue Kinet. 6, 289–301.

169 Wagener, S. and Pfennig, N. (1987) Monoxenic culture of the anaerobic ciliate *Trimyema compressum* Lackey. Arch. Microbiol. 149, 4–11.

170 Wagener, S., Bardele, C.F. and Pfennig, N. (1990) Functional integration of *Methanobacterium formicicum* into the anaerobic ciliate *Trimyema compressum*. Arch. Microbiol. 153, 496–501.

171 Inselburg, J. and Baynal, H.S. (1984) Synthesis of DNA during the asexual cycle of *Plasmodium falciparum* in culture. Mol. Biochem. Parasitol. 10, 79–87.

172 Hare, J.D. (1986) Two-color flow-cytometric analysis of the growth cycle of *Plasmodium falciparum* in vitro: identification of cell cycle compartments. J. Histochem. Cytochem. 34, 1651–1658.

173 Kasper, L.H. and Pfefferkorn, E.R. (1982) Hydroxyurea inhibition of growth and DNA synthesis in *Toxoplasma gondii*: characterization of a resistant mutant. Mol. Biochem. Parasitol. 6, 141–150.

174 Heilporn-Pohl, V. and Limbosch-Rolin, S. (1969) Effects of hydroxyurea on *Acetabularia mediterranea*. Biochim. Biophys. 174, 220–229.

175 Heilporn, V. and Limbosch, S. (1970) Effects of hydroxyurea and ethidium bromide on *Acetabularia mediterranea*. In: Biol. acetabularia, Proc. 1st Int. Symp., 1st (Brachet,J., ed.) pp. 61–72. Academic, New York, NY

176 Howell, S.H., Blaschko, W.J. and Drew, C.M. (1975) Inhibitor effects during the cell cycle in *Chlamydomonas reinhardtii*. Determination of transition points in asynchronous cultures. J. Cell. Biol. 67, 126–135.

177 Bilecka, A. (1979) Effect of hydroxyurea on mitotic activity of [^{3}H]thymidine and [^{3}H]phenylalanine incorporation in the antheridial filament cells of *Charavulgaris*. Acta Soc. Bot. Pol. 48, 179–193.

178 Feller, W. and Follmann, H. (1976) Ribonucleotide reductase activity in green algae. Biochem. Biophys. Res. Comm. 70, 752–758.

179 Feller, W., Schimpff-Weiland, G. and Follmann, H. (1979) Deoxyribonucleotide biosynthesis in synchronous algae cells. Eur. J. Biochem. 110, 85–92.

180 Hofmann, R., Feller, W., Pries, M. and Follmann, H. (1985) Deoxyribonucleotide biosynthesis in green algae. Purification and characterization of ribonucleoside-diphosphate reductase from *Scenedesmus obliquus*. Biochim. Biophys. Acta 832, 98–112.

181 Harder, J. and Follmann, H. (1987) Characterization of the free radical in a plant ribonucleotide reductase. FEBS Lett 222, 171–174.

182 Schimpff, G., Müller, H. and Follmann, H. (1977) Age-dependent DNA and deoxyribonucleotide synthesis in wheat seeds. Biochim. Biophys. Acta 520, 70–81.

183 Hovemann, B. and Follmann, H. (1978) Deoxyribonu-
cleotide synthesis and DNA polymerase activity in plant
cells (*Vicia faba* and *Glycine max*). Biochim. Biophys.
Acta 561, 42–52.

184 Elledge, S.J. and Davis, R.W. (1987) Identification and
isolation of the gene encoding the small subunit of ri-
bonucleotide reductase from *Saccharomyces cerevisiae*:
DNA damage-inducible gene required for mitotic viabil-
ity. Mol. Cell. Biol. 7, 2783–2793.

185 Hurd, H.K., Roberts, Ch.W. and Roberts, J.W. (1987)
Identification of the yeast ribonucleotide reductase small
subunit and its inducibility by methyl methanesulfonate.
Mol. Cell. Biol. 7, 3673–3677.

186 Elledge, S.J. and Davis, R.W. (1990) Two genes differen-
tially regulated in the cell cycle and by DNA-damaging
agents encode alternative regulatory subunits of ribonu-
cleotide reductase. Genes & Devel. 4, 740–751.

187 Yagle, K. and McEntee, K. (1990) The DNA damage-in-
ducible gene *DINI* of *Saccharomyces cerevisiae* encodes a
regulatory subunit of ribonucleotide reductase and is
identical to *RNR3*. Mol. Cell. Biol. 10, 5553–5557.

188 Elledge, S.J., Zhou, Z. and Allen, J.B. (1992) Ribonu-
cleotide reductase: regulation, regulation, regulation.
Trends Biochem. Sci. 17, 119–123.

189 Muller, E.G.D. (1991) Thioredoxin deficiency in yeast
prolongs S phase and shortens the G1 interval of the cell
cycle. J. Biol. Chem. 266, 9194–9202.

190 Harder, J. and Follmann, H. (1990) Identification of a
free radical and oxygen dependence of ribonucleotide
reductase in yeast. Free Rad. Res. Comms. 10, 281–286.

191 Schmidt, W. (1952) Zellvermehrung und Zellatmung bei
Hefe. Z. Naturforsch. 7b, 217–219.

192 Windisch, F., Heumann, W. and Goslich, G. (1953)
Anoxybiotische Zellvermehrung und Wirkungsmodus der
aeroben Wachstums-aktivierung. Z. Naturforsch. 8b,
305–311.

193 Roberts, R.L., Lo, R.J. and Szaniszlo, P.J. (1980) Induc-
tion of synchronous growth in the yeast phase of *Wang-
iella dermatitidis*. J. Bacteriol. 141, 981–984.

194 Land, G.A., Hulme, K.L. and Chaffin, W.L. (1980) The
effect of selected antineoplastic agents on the morphol-
ogy of *Candida albicans* 5865. Can. J. Microbiol. 26,
812–818.

195 Turian, G. and Grange, F. (1976) Anticonidiogenic action
of hydroxyurea in *Neurospora crassa*. C.R. Hebd. Seances
Acad. Sci., Ser. D 282, 169–171.

196 Martegani, E., Tome, F., Trezzi and F., (1981) Timing of
nuclear division cycle in *Neurospora crassa*. J. Cell Sci.
48, 127–136.

197 Carmi, P., Raudaskoski, M., Stamberg, J. and Koltin, Y.
(1977) Meiosis in *Schizophyllum commune*: the effect of
hydroxyurea on basidiospore sporulation, germination,
and nuclear number. Mol. Gen. Genet. 158, 17–21.

198 Raudaskoski, M. and Kaukonen, P. (1978) Effects of
hydroxyurea on development of uninucleate apical cells
of *Schizophyllum commune*. Exp. Mycol. 2, 239–244.

199 Miyata, H., Miyata, M. and Ito, M. (1978) The cell cycle

in the fission yeast, *Schizosaccharomyces pombe*. II. Re-
covery mode of the period of a cycle extended by pulse
treatment with hydroxyurea. Cell Struct. Funct. 3, 153–
159.

200 Lewis, W.H., McNaughton, D.R., LéJohn, H.B. and
Wright, J.A. (1976) Regulation of fungal ribonucleotide
reductase by unusual dinucleotides. Biochem. Biophys.
Res. Comm. 71, 128–135.

201 Stutzenberger, F. (1973) Ribonucleotide reductase of
Pithomyces chartarum: requirement for B_{12} coenzyme. J.
Gen. Microbiol. 81, 501–503.

202 Thelander, L., Gräslund, A. and Thelander, M. (1982)
Continual presence of oxygen and iron required for mam-
malian ribonucleotide reduction: possible regulation
mechanism. Biochem. Biophys. Res. Comm. 110, 859–865.

203 Löffler, M., Schimpff-Weiland, G. and Follmann, H.
(1983) Deoxycytidylate shortage is a cause of G1 arrest of
ascites tumor cells under oxygen deficiency. FEBS Lett.
156, 72–76

204 Löffler, M. (1989) The biosynthetic pathway of pyrimi-
dine (deoxy)nucleotides: a sensor of oxygen tension nec-
essary for maintaining cell proliferation? Exp. Cell Res.
182, 673–680.

205 Sjöberg, B.M., Hahne, S., Mathews, C.Z., Mathews, C.K.,
Rand, K.N. and Gait, M.J. (1986) The bacteriophage T4
gene for the small subunit of ribonucleotide reductase
contains an intron. EMBO J. 5, 2031–2036.

206 Tseng, M.J., Hilfinger, J.M., Walsh, A. and Greenberg,
G.R. (1988) Total sequence, flanking regions, and tran-
scripts of bacteriophage T4 *nrdA* gene, coding for a chain
of ribonucleoside diphosphate reductase. J.Biol. Chem.
263, 16242–16251.

207 Ingemarson, R., Gräslund, A., Darling, A. and The-
lander, L. (1989) Herpes simplex virus ribonucleotide
reductase: expression in *Escherichia coli* and purification
to homogeneity of a tyrosyl free radical-containing, enzy-
matically active form of the 38-kilodalton subunit. J.
Virol. 63, 3769–3776.

208 Prince, R.C. and George, G.N. (1990) Tryptophan radi-
cals. Trends Biochem. Sci. 15, 170–172.

209 Ito, N., Phillips, S.E.V., Stevens, C., Ogel, Z.B., McPher-
son, M.J. Keen, J.N., Yadav, K.D.S. and Knowles, P.F.
(1991) Novel thioether bound revealed by a 1.7 Å crystal
structure of galactose oxidase. Nature 350, 87–90.

210 Prince, R.C. (1988) Tyrosine radicals. Trends Biochem.
Sci. 13, 286–288.

211 Kerscher, L. and Oesterhelt, D. (1982) Pyruvate:ferredo-
xin oxidoreductase – new findings on an ancient enzyme.
Trends Biochem. Sci 7, 371–374.

212 Ortiz De Montellano, P.R. (1987) Control of the catalytic
activity of prosthetic heme by the structure of hemopro-
teins. Acc. Chem. Res. 20, 289–294.

213 Ghisla, S. and Massey, V. (1989) Mechanisms of flavopro-
tein-catalyzed reactions. Eur. J. Biochem. 181, 1–17.

214 Frey, P.A. (1989) 2-acetylthiamin pyrophosphate: an en-
zyme-bound intermediate in thiamin pyrophosphate-de-
pendent reactions. BioFactors 2, 1–9.

215 Rétey, J. (1990) Enzymic reaction selectivity by negative catalysis or how do enzymes deal with highly reactive intermediates? Angew. Chem. Int. Ed. Engl. 29, 355–361.

216 Karthein, R., Dietz, R., Nastainczyk, W. and Ruf, H.H. (1987) Higher oxidation states of prostaglandin H synthase Eur. J. Biochem. 171, 313–320.

217 Shimokawa, T., Kulmacz, R.J., DeWitt, D.L. and Smith, W.L. (1990) Tyrosine 385 of prostaglandin endoperoxide synthase is required for cyclooxygenase catalysis. J. Biol. Chem. 265, 20073–20076.

218 Abeles, R.H. and Dolphin, D. (1976) The vitamin B_{12} coenzyme. Acc. Chem. Res. 9, 114–120.

219 Halpern, J. (1985) Mechanisms of coenzyme B_{12}-dependent rearrangements. Science 227, 869–875.

220 Frey, P.A. (1990) Importance of organic radicals in enzymatic cleavage of unactivated C-H bonds. Chem. Rev. 90, 1343–1357.

221 Hartmanis, M.G.N. and Stadtman, T.C. (1986) Diol metabolism and diol dehydratase in Clostridium glycolicum. Arch. Biochem. Biophys. 245, 144–152.

222 Hartmanis, M.G.N. and Stadtman, T.C. (1987) Solubilization of a membrane-bound diol dehydratase with retention of EPR g = 2.02 signal using 2-(N-cyclohexylamino)-ethanesulfonic acid buffer. Proc. Natl. Acad. Sci. USA 84, 76–79.

223 Lippard, S.J. (1988) Oxoverbrückte Polyeisenzentren in Biologie und Chemie. Angew. Chem. 100, 353–371.

224 Vincent, J.B., Olivier-Lilley, G.L. and Averill, B.A. (1990) Proteins containing oxo-bridged dinuclear iron centers: a bioinorganic perspective. Chem. Rev. 90, 1447–1467.

225 Mathews, C.K., Moen, L.K., Wang, Y. and Sargent, R.G. (1988) Intracellular organization of DNA precursor biosynthetic enzymes. Trends Biochem. Sci. 13, 394–397.

226 Young, J.P. and Mathews, C.K. (1991) Interactions between T4 phage-coded deoxycytidylate hydroxymethylase and thymidylate synthase as revealed with an anti-idiotypic antibody. J. Biol. Chem. 267, 10786–10790.

227 Mountfort, D. and Rhodes, L.L. (1991) Anaerobic growth and fermentation characteristics of Paecilomyces lilacinus isolated from mullet gut. Appl. Environ. Microbiol. 57, 1963–1968.

228 Breton, A., Dusser, M., Gaillard-Martinie, B., Guillot, J., Millet, L. and Prensier, G. (1991) Piromyces rhizinflata nov. sp., a strictly anaerobic fungus from faeces of the saharian ass: a morphological, metabolic and ultrastructural study. FEMS Mircobiol. Lett. 82, 1–8.

229 Teunissen, M.J., Op den Camp, H.J.M., Orpin, C.G., Huis in 't Veld, J.H.J. and Vogels, G.D. (1991) Comparison of growth characteristics of anaerobic fungi isolated from ruminant and non-ruminant herbivores during cultivation in a defined medium. J. Gen. Microbiol. 137, 1401–1408.

230 Marvin-Sikkema, F.D., Lahpor, G.A., Kraak, M.N., Gottschal, J.C. and Prins, R.A. (1992) Characterization of an anaerobic fungus from lama faeces. J. Gen. Microbiol. 138, 2235–2241.

231 Cai, J., Speed, R.R. and Winkler, H.H. (1991) Reduction of ribonucleotides by the obligate intracytoplasmic bacterium Ricksettsia prowazekii. J. Bacteriol. 173, 1471–1477.

232 Bornemann, C. and Follmann, H. (1993) Deoxyribonucleotide synthesis in phycovirus-infected green algae. A new virus-induced ribonucleotide reductase. Z. Naturforsch. 48c, 113–117.

233 Eschenmoser, A. (1988) Vitamin B_{12}: experiments concerning the origin of its molecular structure. Angew. Chem., Int. Ed. Engl. 27, 5–39.