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Forum Review

Ribonucleotide Reductases: Influence of Environment on Synthesis and Activity

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ABSTRACT

Ribonucleotide reductases (RNRs) are enzymes that provide deoxyribonucleotides (dNTPs), the building blocks required for *de novo* DNA synthesis and repair. They are found in all organisms from prokaryotes to eukaryotes. Interestingly, in the microbial world, several organisms possess the genes encoding two, or even three different RNRs that present different structures and allosteric regulation. The finding of an increasing number of bacterial species that possess more than one RNR might suggest particular functions for these enzymes in different growth conditions. Recent support for this proposal comes from studies indicating that expression and activity of the different RNRs depends on the environment. The oxygen content as well as the redox and oxidative stresses regulate RNR activity and synthesis in various organisms. This regulation has a direct consequence on dNTP pools. An excess of dNTP pools that leads to misincorporation of dNTPs results in genetic abnormalities in eukaryotes as in prokaryotes. In contrast, increased dNTP concentrations help cells to survive under conditions where DNA has been damaged. Hence the use of different RNRs in response to various environmental conditions allows the cell to regulate the amount precisely of dNTP in both a positive and negative manner so that enough, yet not excessive, dNTPs are synthesized. *Antioxid. Redox Signal.* 8, 773–780.

INTRODUCTION

Ribonucleotide reductases (RNRs) are enzymes that provide deoxyribonucleotides (dNTPs), the building blocks required for *de novo* DNA synthesis and repair (15, 31, 59). They are found in all organisms from prokaryotes to eukaryotes. RNRs use radical chemistry to create an activated protein cysteinyl radical (S•) that catalyzes the reduction of nucleotides by abstracting a hydrogen atom from the ribose ring of the ribonucleotides (53). Appearance of this cysteinyl radical requires the prior generation of either a tyrosyl or a glycyl radical, depending on the type of RNR. Based on the mechanism used to create this radical in their active site, RNRs are divided into three classes (class I, II, and III) (10, 48, 54, 55).

To generate the cysteinyl radical, class I RNRs use a tyrosyl radical that is itself generated by a diferric center together with oxygen (36). Thus, class I reductases depend on molecular oxygen for their activity (19, 61). They are present in all higher organisms, in certain viruses, and in eubacteria, but are not present in archaea. Class I RNRs are further divided into two subclasses (Ia and Ib) based on allosteric regulation and structural differences. Class Ia RNRs are composed of two nonidentical subunits, R1 and R2. In *Escherichia coli*, R1 and R2 are expressed coordinately from the *nrdA* and *nrdB* genes, respectively, which are located in an operon (23). The activity of the holoenzyme is controlled by a complex feedback regulation through binding of allosteric effectors to R1. This allosteric regulation of RNR activity has been extensively studied (11, 17). Class Ib RNRs are widespread among eubacteria. They possess the same structure and metal center as the class Ia enzymes (the corresponding R1 and R2 proteins are encoded by the *nrdE* and *nrdF* genes, respectively), but they share only modest se-

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quence identity with class Ia RNRs and also differ in some functional aspects, especially the allosteric regulation. Class II RNRs also use a tyrosyl radical, but it is generated by an adenosylcobalamin cofactor (vitamin B12) (4). Class II RNRs, are composed of a monomeric protein of 82 kDa that functions in an equivalent manner to protein R1 (3, 44). The best-characterized class II enzyme is that from *Lactobacillus leichmannii,* and is encoded by the *nrdJ* gene. Class II RNRs exhibit significant amino acid sequence identity with the R1 subunit of class I enzymes (3). They share with the class I enzymes conservation of the functional cysteines, as well as some features of the allosteric regulation of substrate specificity (13). However, since the mechanism that generates the tyrosyl radical does not depend on the presence of oxygen, the reduction of ribonucleotides by class II reductases occurs equally well under both anaerobic and aerobic conditions. Class II RNRs are widespread among aerobic and anaerobic eubacteria and archaea.

Class III enzymes use a glycyl radical instead of the tyrosyl radical found in class I and II RNRS. The glycyl radical of this group of enzymes is generated by a complicated activation reaction requiring S-adenosylmethionine (SAM) and an [4Fe–4S] iron–sulfur cluster (40). The extreme oxygen sensitivity of this radical limits the function of class III enzymes to bacteria growing in the strict absence of oxygen (42). The *E. coli* NrdDG enzyme is the prototype for all class III RNRs.

Electrons required for the ribose reduction in RNRs are supplied by different donors: formate for the anaerobic class III enzyme (41) and the small thiol-redox proteins, thioredoxin (Trx) or glutaredoxin (Grx) for class I and class II reductases (4, 25, 53, 58). Thioredoxins and glutaredoxins, which belong to the thioredoxin superfamily, are defined by a common "thioredoxin fold" and a conserved active site that most often contains a cys-x-x-cys sequence involved in the redox reactions (25) (for review, see Ref. 49). While the glutaredoxins and thioredoxins exhibit similar three-dimensional structures and active sites, two different enzymatic systems keep them in a reduced state. The pathway for the reduction of oxidized glutaredoxins initially utilizes NADPH which maintains the enzyme glutathione reductase in the reduced state. This latter enzyme, in turn, transfers electrons to disulfide glutathione generating glutathione, which then transfers electrons to oxidized glutaredoxins. In the case of the thioredoxins, the initial source of electrons is also NADPH; but, in this case the electrons are transferred to the enzyme thioredoxin reductase which then directly reduces oxidized thioredoxins (for review, see Refs. 25, 49).

Interestingly, in the microbial world, several organisms possess the genes encoding two, or even three different RNRs (32). The finding of an increasing number of bacterial species that possess more than one RNR might suggest particular functions for these enzymes in different growth conditions. Recent support for this proposal comes from studies indicating that expression and activity of the different RNRs depends on the redox environment. In this review we will focus on the effect of the oxygen content, the redox environment, and oxidative stress on RNR activity and synthesis (and therefore on dNTP pools).

INFLUENCE OF OXYGEN CONTENT ON RNR ACTIVITY AND SYNTHESIS

Evolution of RNR with appearance of molecular oxygen

The appearance of oxygen during evolution, which drastically changed the conditions for the radical-based reaction mechanism, may explain the evolution of an original RNR into the three classes of RNR present today in living organisms. For organisms that confronted aerobic conditions, mutational alterations that changed the anaerobic RNR so that it could function in this very different environment were likely selected. For those facultative aerobic bacteria that encountered both aerobic and anaerobic environments, these alterations could have resulted in two different enzymes, one for each environment, or a single enzyme that could function both aerobically and anaerobically.

It would also have been advantageous to develop regulatory mechanisms that controlled the expression or activity of the aerobic and anaerobic RNRs depending on the presence or absence of oxygen. In *Escherichia coli*, an aero–anaerobic facultative bacterium, the anaerobic class III RNR, NrdDG, is oxygen-sensitive. Exposure of the enzyme to oxygen results in proteolytic cleavage adjacent to the glycyl radical and removal of the carboxy-terminal 25 residues, leading to inactivation of the enzyme. Class III enzymes which exhibit this regulation are functional only in strict anaerobiosis; they are present in strict anaerobes and in certain facultative anaerobes only during growth under anaerobic conditions (33). In contrast, the two class I RNRs, such as the *E. coli* NrdAB and NrdEF, require molecular oxygen for their activity (Table 1).

TABLE 1. OXYGEN DEPENDENCY OF *E. COLI* RIBONUCLEOTIDE REDUCTASES; ABILITY OF SPECIFIC RIBONUCLEOTIDE REDUCTASES TO FUNCTION IN PRESENCE OF VARIOUS AMOUNTS OF OXYGEN *IN VIVO*

	Aerobiosis	*Microaerobiosis*	*Anaerobiosis*
Class Ia (NrdAB)	+	+	−
Class Ib (NrdEF)	+ (when overexpressed)	−	−
Class III (NrdDG)	−	−	+

The relationship between oxygen content and RNR activity observed *in vitro* has been confirmed *in vivo* by genetic studies. The *nrdD* and *nrdG* genes are essential in strict anaerobiosis; *nrdDG* defective mutants are not viable in the absence of oxygen. These results indicate that the only functional RNR in strict anaerobiosis in *E. coli* is NrdDG (22). In contrast, class I RNRs, which require molecular oxygen for radical formation, function only under aerobic conditions (19). The *nrdA* and *nrdB* genes are essential for aerobic growth; *nrdAB* defective mutants are not viable in the presence of oxygen. The *nrdAB* defective strain is not rescued by the presence of the *nrdEF* genes which encodes the class Ib RNR, as these genes are not expressed under normal laboratory growth conditions. Thus, the only functional RNR in aerobiosis in *E. coli* is NrdAB (30). However, recent studies show that when overexpressed from a plasmid, *nrdHIEF* can suppress the growth defect of an *nrdAB* mutant (30) (Gon *et al.,* unpublished observations). (NrdH and NrdI are accessory genes encoded within the same operon as NrdEF, which will be described later in this review.) In contrast to the *nrdAB* genes, the *nrdEF* operon is not essential to the cells; *nrdEF* defective mutants are viable under both aerobic and anaerobic conditions (30).

Another difference between the class Ia NrdAB and the Class Ib NrdEF is that the amounts of oxygen required for their activity differ in *E. coli*. In microaerobiosis (GasPack anaerobic system, BBL) where NrdDG is the major source of dNTPs, expression of *nrdAB* can weakly substitute for growth when *nrdDG* is deleted. In contrast, *nrdEF* does not suffice for growth in microaerobiosis, even when the genes are overexpressed from an inducible plasmid (22) (Gon *et al.,* unpublished observations). In accordance with their biological function and their dependence on the molecular oxygen content, the expression of class I and class III enzymes (the *nrdAB, nrdEF,* and *nrdDG* operons) is also influenced by the oxygen content in the environment (Table 1). When *E. coli* is shifted from aerobic to anaerobic conditions, the transcription of the *nrdDG* operon is strongly induced while *nrdAB* transcription is turned off (6, 8, 22). This shift results in major changes in gene expression, orchestrated by two regulatory systems, the iron–sulfur FNR protein and the ArcA and ArcB products (27, 51). These two regulatory systems have different affinities for oxygen, thereby allowing the detection of different concentrations of oxygen which can repress or activate transcription of various genes. In anaerobiosis, the induction of *nrdDG* is completely dependent on the global transcriptional regulator FNR but is independent of the ArcA–ArcB two-component system (6). An Fnr box has been found upstream of the *nrdDG* operon (57). Moreover, the transcription of the *nrdAB* operon is decreased at low oxygen tension (8), suggesting involvement of the ArcAB system. The two systems appear to set the activities of the *nrdAB* and *nrdDG* operons in relation to the availability of oxygen. In contrast, expression of the *nrdEF* operon is not affected by oxygen availability (6).

Ribonucleotide reductase in other bacteria

The multiplicity of RNRs has also been observed in several other organisms. The *Staphyloccocus aureus* genome contains two gene clusters, one resembling *nrdEF* (class Ib) and another resembling *nrdDG* (class III). In this organism, expression of genes coding for these aerobic and anaerobic RNRs, is also regulated in response to oxygen concentration. *S. aureus* adapts to changes in oxygen concentration by modulating the transcriptional regulation of genes that encode the aerobic class Ib and anaerobic class III ribonucleotide reductase (RNR) systems. Similarly to *E. coli*, transcription of the class III *nrdDG* genes is regulated by oxygen concentration and is at least 10-fold higher under anaerobic than under aerobic conditions. In contrast, no significant effect of oxygen concentration is found on the transcription of class Ib *nrdIEF* genes (38).

Streptomyces coelicolor also contains the genetic information for two separate ribonucleotide reductases, one oxygen-independent class II RNR (*nrdJ*) and one oxygen-dependent class Ia (*nrdAB*). Borovok and co-workers showed that *S. clavuligerus* class Ia and class II RNR genes were differentially transcribed during vegetative growth (5). The copy number of the class II *nrdJ* transcripts was approximately constant throughout the exponential phase of vegetative growth. In contrast, transcription of the class Ia encoding genes (*nrdAB*) was 10- to 20-fold less than that of *nrdJ* in the early-exponential growth phase, and decreased even more during mid-exponential and late-exponential phases of growth (5). Based on these results, the authors propose that streptomycetes employ two RNRs: a class Ia oxygen-dependent RNR and a class II oxygen-independent RNR that function at different stages in the growth cycle. The class Ia RNR might operate primarily in the early stages of growth following spore germination, whereas the class II RNR might act primarily during vegetative growth. Vegetative growth of *Streptomyces* spp. occurs mainly by cell-wall extension at hyphal tips, with lateral branching leading to high cells density. The younger and older parts of the mycelium are not physically homogeneous and may be subject to different degrees of oxygen availability. Thus, the existence of two classes of RNRs that differ in their dependence on oxygen may be necessary for proper growth and development. In accordance with this model, presence of *nrdJ* stimulates recovery of growth of *Streptomyces* after oxygen limitation. Therefore, the class II RNR (NrdJ) may function to provide a pool of deoxyribonucleotide precursors for DNA repair during oxygen limitation and/or for immediate growth after restoration of oxygen (5).

Surprisingly, some strict anaerobes such as *Bacteroides fragilis* also contain the genetic information for class I oxygen-dependent RNRs. However, although *B. fragilis* is an obligate anaerobe, it is capable of long-term survival in the presence of air. Survival is attributed to an elaborate oxidative stress response that controls the induction of more than 28 proteins included among which is the *Bacteroides* NrdAB (50). It may be that during aerobic conditions *B. fragilis* NrdAB has a role in maintaining dNTPs pools for DNA repair and growth recovery.

INFLUENCE OF OXIDATIVE STRESS ON RNR ACTIVITY AND SYNTHESIS

The class Ib RNRs, which includes NrdEF, were discovered in enterobacteria decades after the detection of the

NrdAB ribonucleotide reductase (29). This enzyme class was first identified in a screen using a plasmid library of *Salmonella typhimurium* to complement a temperature-sensitive *nrdA* mutant of *E. coli* (28). Since that discovery, it has become clear that the class Ib enzymes, rather than class Ia, represent the essential ribonucleotide reductase in many bacteria (31), in contrast to *E. coli* where *nrdEF* operon is not usually expressed in sufficient amounts to support growth (30) (Gon *et al.,* unpublished observations). Neither inhibitors of DNA replication nor DNA damage, which induce expression of *nrdAB,* cause increased expression of the *nrdEF* genes in *E. coli.* Thus, until recently, the *E. coli* NrdEF was considered a cryptic enzyme with no obvious function (30). The existence of this new class Ib of the centrally important RNR enzymes in *E. coli*, where a class Ia already exists, raises questions about its role in the bacteria. Why would two aerobic class I enzymes be retained in the organism?

A possible role for NrdEF is suggested by studies of Monje-Casas *et al.* (39) who found that expression of the *nrdHIEF* operon in *E. coli* increased under oxidative stress conditions. Oxidative stress occurs when, during aerobic respiration, detrimental reactive oxygen species (ROS) such as H_2O_2, superoxide anion, hydroxyl radical, and alkyl hydroperoxides are produced and cause damage to proteins, nucleic acids, and the lipids of cell membranes (26). The expression of *nrdHIEF* is triggered in bacteria exposed to increasing concentrations of different oxidants such as hydrogen peroxide or Paraquat (PQ) (a superoxide-generating compound). Expression of *nrdHIEF* is also increased in mutant bacteria compromised in major defenses against oxidative stress, such as catalase, superoxide dismutase, and alkyl hydroperoxide reductase activities, which catalyze destruction of hydrogen peroxide or alkyl hydroperoxides (20, 39, 60). Increases in *nrdHIEF* expression as high as 70-fold have been observed in some of these conditions. In contrast, the expression of the *nrdAB* operon that codes for the main class I reductase is not induced by oxidative stress (39, 45) (Table 2). The mechanism that triggers *nrdHIEF* expression under oxidative stress conditions is not known. Studies on this stress response indicate that the presence of reactive oxygen species must be sensed by regulators that are distinct from key global regulators usually implicated in the adaptive response to H_2O_2 and superoxide radicals, OxyR and SoxRS, respectively (recently reviewed in Ref. 52).

Allosteric regulation rapidly adapts an enzyme to changing requirements for its product by binding of effectors that increase or decrease its activity. Most class Ia reductases are regulated in this way by binding either ATP (activating) or dATP (inhibitory) to the activity site of protein R1 (7). However, reductases also have an additional and unique allosteric mechanism that regulates their substrate specificity (specificity site) and ensures that the enzyme produces equal amounts of each dNTP for DNA synthesis. Disturbances in pool sizes lead to genetic damage and in severe cases to cell death (reviewed in Ref. 35). Such disasters are prevented by binding of end products (dATP, dGTP, and dTTP) to the specificity site of the reductases (31). Class Ib (12) and class II (13) enzymes harbor only specificity sites, and therefore these enzymes are not inhibited by dATP (12, 31, 37).

The homodimeric NrdEF enzyme consists of two tightly bound proteins, R1E and R2F. Nucleoside triphosphates (ATP, dATP, dGTP, and dTTP) regulate the substrate specificity by binding to a single site on the R1E protein. This regulation is similar to that of the NrdAB enzyme. However, in contrast to NrdE, the NrdA protein contains a second binding site for dATP (and ATP) that controls general enzyme activity and binding of dATP to this site strongly inhibits all activity of the NrdAB enzyme.

One major difference between class Ia and class Ib enzymes is that NrdE proteins lack approximately 50 amino-terminal residues present in the R1 protein of the class Ia enzyme that correspond to the site that responds to allosteric effectors (31). As a consequence, class Ib enzymes are not inhibited by dATP. The more sophisticated regulation of NrdAB enzymes may provide protection against the possible harmful overproduction of dNTPS (see below). At the same time one could imagine that the lack of a negative feedback mechanism for inhibition of NrdEF could be responsible for higher dNTPs production in conditions when higher dNTPS levels are required for DNA repair (see below).

A second advantage for *E. coli* in using the NrdEF RNR instead of NrdAB during oxidative stress is that the *nrdAB* operon is controlled by the transcriptional factor DnaA, whereas *nrdHIEF* may not be (there are no DnaA boxes in the *nrdHIEF* promoter region in contrast to *nrdAB*). The transcription of the *nrdAB* operon is repressed in conditions where an excess of dNTPs is synthesized and this negative feedback appears to act through the regulatory activity of DnaA (Gon *et al*., unpublished observations). It may be that binding of dNTPs to DnaA activates it as a repressor. Since the *nrdHIEF* promoter region does not contain any DnaA boxes, the expression of *nrdHIEF* may not be downregulated by the products (dNTPs) of the RNR when higher dNTP levels are required for DNA repair.

TABLE 2. GENETIC AND ALLOSTERIC REGULATION OF *E. COLI* RIBONUCLEOTIDE REDUCTASES; INFLUENCE OF ENVIRONMENTAL STRESSES ON GENE EXPRESSION

	Genetic regulation	*Allosteric regulation (dATP inhibition)*
Class Ia (NrdAB)	Induction by thiol-redox stress	
	Induction by DNA damage	+
	Induction by DNA replication inhibitors	
Class Ib (NrdEF)	Induction by oxidative stress	−
	Induction by thiol-redox stress	

INFLUENCE OF REDOX STRESS ON RNR ACTIVITY AND SYNTHESIS

Upregulation of ribonucleotide reductase in mutants lacking thioredoxins and glutaredoxins

Since thioredoxins or glutaredoxins are required for the regeneration of active RNR under aerobic conditions, modulation of the cytoplasmic redox environment (redox stress) can modulate the activity of RNR. In *E. coli*, there are two major thioredoxins, thioredoxin 1 (*trxA*) and thioredoxin 2 (*trxC*), and three glutaredoxins, glutaredoxin 1 (*grxA*), glutaredoxin 2 (*grxB*), and glutaredoxin 3 (*grxC*). Of these, only the enzymes glutaredoxin 1, thioredoxin 1, and thioredoxin 2 are capable of reducing the essential class Ia enzyme RNR to regenerate its activity *in vivo* and *in vitro* (2, 46). Under usual laboratory growth conditions, however, thioredoxin 2 is not produced in sufficient quantities to reduce NrdAB efficiently. Effects of deletion of the genes for some of these proteins leads to a modification of the transcription of the *nrdAB* and *nrdEF* operons (45). This is seen as an inverse relationship between expression of *nrdAB* and of genes encoding components of the glutaredoxin (*grxA* and *gor*) and thioredoxin (*trxA* and *trxB*) pathways (45). A *trxA, grxA* double mutant exhibits a >20-fold increase in the expression of NrdAB, presumably to restore the normal levels of dNTP synthesis that is normally maintained by thioredoxin 1 and glutaredoxin 1. This regulation operates at the transcription level (21). However, *nrdAB* derepression is not mediated by OxyR, a regulatory protein that responds to oxidative stress, even though OxyR is often activated by defects in these redox pathways (62). Dramatically increased increments in the steady-state levels of *nrdHIEF* transcripts (100-fold) were also detected in mutants simultaneously lacking both the Trx- and Grx/GSH-reducing pathways, the two main reductants of the NrdAB reductase (39) (Table 2). However, the increased level of NrdHIEF in these conditions cannot compensate for the lack of the *nrdAB* operon.

Higher levels of RNR can compensate for a lack of certain thioredoxins and glutaredoxins

Two questions arise concerning the observed upregulation of the *nrdAB* and the *nrdHIEF* operons in the absence of Trx and Grx components: does this upregulation of the two aerobic RNRs ensure viability of the defective cells and what is the mechanism of this regulation? Recent results from our laboratory provide some answers to these questions. Our insights into these issues come from studies of suppressor mutations that restore growth to strains lacking several of the thioredoxins and glutaredoxins. A strain deleted for genes encoding four reductants (TrxA, TrxC, GrxA, and NrdH) is unable to grow, presumably due to the accumulation of oxidized NrdAB and the resultant lowering of dNTP production (43). From this multiply defective strain, three extragenic suppressor mutations were characterized that restored growth. Two of the mutations mapped to the *dnaA* gene and one to the *dnaN* gene, genes whose protein products are essential for DNA replication. The suppressor mutations cause five- to eightfold increases in the expression of the *nrdAB* genes. The results indicate that the increased levels of the ribonucleotide reductase, NrdAB, are sufficient to restore growth, since growth can also be restored to the mutant strain simply by overexpressing NrdAB from a plasmid. In both cases, derepression of NrdAB via the *dnaA* or *dnaN* mutations or overexpression of NrdAB from a plasmid, the restoration of growth is dependent on one of the remaining glutaredoxins, glutaredoxin 3. Apparently, glutaredoxin 3 which exhibits only a low capacity to reduce oxidized NrdAB *in vivo* and *in vitro*, provides sufficient reductive power when high levels of NrdAB are achieved. This result, in conjunction with results from other laboratories (see below), suggests that a mechanism of regulation that increases the levels of RNR when cells are deficient in their reductive pathways is important to the bacteria. This mechanism, which we observe under conditions where the redox components are absent due to mutations, would also come into play when the cells are subjected to redox stress. The mechanism of regulation of NrdAB by the *dnaA* and *dnaN* mutations has also been elucidated by these studies (Gon *et al*., submitted for publication). DnaA, which is essential for initiation of DNA replication, is also a transcriptional regulatory protein that represses NrdAB expression. The *dnaA* suppressor mutations alter the ATP-bound state of DnaA, thus causing derepression of NrdAB. Analysis of the properties of these mutant proteins suggest that the regulatory activity of DnaA confers on the cell an important mechanism coordinating initiation of DNA replication and the synthesis of pools of dNTPs during the cell cycle. These studies also show that expression of *nrdAB* is controlled by the pool of dNTPs itself. Overexpression of RNRs in *E. coli* leads to higher dNTPs pools and also leads to repression of the *nrdAB* operon. This control also operates through DnaA. Thus, variations in the interactions between cellular redox components, ribonucleotide reductase and protein components of the DNA replication machinery may be important in the response to oxidative stress as well as under normal growth conditions.

INFLUENCE OF VARIATION IN RNR SYNTHESIS AND ACTIVITY ON DNTP POOLS

Consequence on genome integrity

Considerable evidence exists for the cell cycle regulation of the *nrdAB* operon (56). The amount of RNR protein, and thus, deoxyribonucleotides, is coordinated with the rate of DNA synthesis, which is determined by replication initiation events. In an *E. coli* cell growing with a generation time longer than the time required for replication of the chromosome, the need for RNR goes from zero to a maximum as DNA initiation occurs. NrdAB mRNA synthesis increases at approximately the time at which DNA initiation occurs (18, 24), a finding similar to those observed in eukaryotic organisms (1, 14, 16). This regulatory mechanism generates a controlled burst of RNR synthesis every time a new replication fork is initiated and ensures the appropriate amount of enzyme under any growth condition. Therefore, the amount of dNTPs present at any given moment during the cell cycle would appear to be enough for just a few minutes of DNA

replication. A balanced pool of the deoxyribonucleotide triphosphate building blocks, the precursors of DNA is a fundamental requirement for these processes. Synthesis of deoxyribonucleotides is an extremely well-regulated reaction. A failure in the control of the dNTP levels and/or their relative amounts leads to cell death or genetic abnormalities in eukaryotes as in prokaryotes (9, 47) (Gon *et al.*, submited observations). Indeed, a lack of RNR activity (either in a *nrdDG* mutant in strict anaerobiosis or in a *nrdAB* mutant in aerobiosis) leads to cell death (43) (Gon *et al.*, submitted). Why does *E. coli* exhibit such complicated regulation of dNTP synthesis and not simply maintain excess dNTPs throughout the cell cycle? An argument could be made simply on the basis of efficiency; overproduction of dNTPs is wasteful. However, studies in yeast and more recently in bacteria, point to another reason. An excess of dNTP pools is responsible for misincorporation of dNTPs which results in DNA mutagenesis due to increased error rates of DNA polymerases. dNTP pools may have to be regulated to avoid deleterious mutagenic effects on the cells (9, 34). In contrast to the need to avoid high amounts of dNTPs in normal growth conditions, in yeast, increased dNTP concentrations helps cells to survive under conditions where DNA has been damaged (9). Therefore, the expansion of dNTP pools may have an important physiological role during DNA damage. Chabes *et al.* (9) have speculated that increased dNTP concentration allows more efficient bypass of DNA lesions by DNA polymerases. Regulatory mechanisms that cause increased synthesis of dNTPs by upregulating or enhancing the activity of an RNR could come into play under stress conditions that lead to DNA damage. For example, in *E. coli*, the *nrdHIEF* operon is upregulated during oxidative stress in contrast to *nrdAB* which encodes the main RNR. This induction leads to an increase of RNR that lacks the negative feedback allosteric regulation exhibited by the class Ia RNR (NrdAB), and thus also presumably to increased dNTP pool. Therefore, in a similar way to what have been observed in yeast, a relaxed negative control of RNR activity could help cells to survive under conditions where DNA has been damaged.

CONCLUSIONS

Ribonucleotide reductases (RNRs) play a crucial role in all organisms since they provide deoxyribonucleotides (dNTPs), the building blocks required for both DNA replication and repair. They are found in all organisms from prokaryotes to eukaryotes, and in the microbial world, several organisms possess several RNRs. Microorganisms possess at least two ways they can respond to change in environment (e.g., aerobic, aerobic) or to environmental stress (e.g., redox and oxidative stress): (a) turning on different RNRs at different times; or (b) activating a number of regulatory mechanisms that vary the amounts and activities of these enzymes. Some of these mechanisms are important for the cell cycle as dNTPs must be synthesized in coordination with DNA replication at particular times during the cell cycle. However, we are only at the beginning of understanding these regulatory mechanisms. Many questions regarding the molecular mechanism of these regulations remain: Are there specific regulatory proteins that control theexpression of RNRs synthesis in conditions of redox and oxidative stress? Are there any direct effects of redox or oxidative conditions on regulatory proteins or on RNR?

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ABBREVIATIONS

dNTP, deoxyribonucleotide triphosphate; Grx, glutaredoxin; RNR, ribonucleotide reductase; SAM, S-adenosylmethionine; Trx, thioredoxin.

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