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Review

Cellular regulation of ribonucleotide reductase in eukaryotes

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ABSTRACT

Synthesis of deoxynucleoside triphosphates (dNTPs) is essential for both DNA replication and repair and a key step in this process is catalyzed by ribonucleotide reductases (RNRs), which reduce ribonucleotides (rNDPs) to their deoxy forms. Tight regulation of RNR is crucial for maintaining the correct levels of all four dNTPs, which is important for minimizing the mutation rate and avoiding genome instability. Although allosteric control of RNR was the first discovered mechanism involved in regulation of the enzyme, other controls have emerged in recent years. These include regulation of expression of RNR genes, proteolysis of RNR subunits, control of the cellular localization of the small RNR subunit, and regulation of RNR activity by small protein inhibitors. This review will focus on these additional mechanisms of control responsible for providing a balanced supply of dNTPs.

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1. Introduction

Ribonucleotide reductases (RNR) are key enzymes in all organisms essential for the *de novo* synthesis pathway of deoxyribonucleoside triphosphates (dNTPs), required for DNA replication and repair. They are of particular interest as their activity largely determines the concentrations and ratios of dNTPs and these factors are critical in ensuring high-fidelity DNA synthesis [1–6]. High concentrations of dNTPs reduce the efficiency of polymerase proofreading, but may also serve to facilitate repair by promoting the ability of polymerases to copy damaged template. Imbalances in dNTP levels reduce the fidelity of the initial polymerization step and even subtle defects can be highly mutagenic [7]. Inhibition of RNR slows DNA replication and activates the intra-S phase checkpoint, which helps to preserve limiting dNTPs [8,9]. If the S phase checkpoint is inactive, DNA synthesis is not restrained by limiting dNTPs and ongoing replication leads to DNA damage and cell death [10]. Failure to upregulate dNTP levels during cell proliferation has been shown to promote oncogene-induced transformation, emphasizing the importance of RNR regulation for genome stability [11]. The key role that RNR has in cell proliferation is exploited in chemotherapy of several types of cancer, using inhibitors such as hydroxyurea, clofarabine and gemcitabine [12,13].

The cellular pool of dNTPs is sufficient for replication of just a fraction of the genome, so upregulation of RNR activity is necessary

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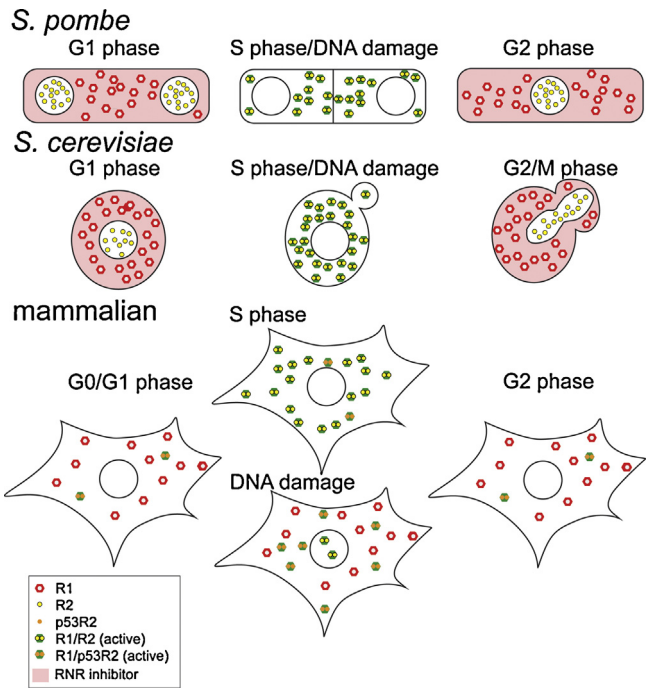

Fig. 1. Overview of cell cycle and DNA damage induced changes in RNR regulation in yeasts and mammalian cells. No attempt is made to depict accurately the cellular localization of small protein RNR inhibitors. Note that in *S. pombe*, S phase normally occurs before cytokinesis is complete, so G1 and S phase cells are shown binucleated.

as cells enter S phase. Allosteric mechanisms regulate the activity and specificity of RNR but a wider range of cellular mechanisms impinge on the enzyme. These include altering the expression or proteolysis of RNR subunits, changing the cellular localization of individual subunits, or altering the levels of small protein inhibitors to control the enzyme activity (Fig. 1). In *Saccharomyces cerevisiae*, a combination of regulatory mechanisms serve to elevate the dNTP levels several fold in S phase or after DNA damage [2], although in *Schizosaccharomyces pombe* a much more modest increase is seen [14] (Table 1). Mammalian cells show an even more dramatic elevation of dNTP levels in S phase, although curiously little change after DNA damage ([15], reviewed in [16]). Allosteric regulation of RNR has been the subject of recent reviews [17,18] and will only be summarized here, and the purpose of this review is to consider recent developments in cellular regulatory mechanisms.

2. Outline of RNR structure and biochemistry

RNRs are classified into three main classes depending on the mechanism of free radical generation, which is essential for catalysis (reviewed in [19]). Class I RNRs are aerobic enzymes composed of two subunits, and are further subdivided depending on the metallocofactor used. Eukaryotes predominantly use Class 1a RNRs, which have a Fe(III) metallocofactor in the smaller subunit (R2, β) and a catalytic site in the larger subunit (R1, α). The R2 subunit generates and stabilizes a tyrosyl radical, which creates a reactive cysteine thyil radical in the active site necessary for initiating catalysis (reviewed in [18,20]). During a reaction cycle, a disulphide bond is generated in the R1 subunit which must be reduced by thioredoxin or glutaredoxin to reactivate the enzyme. This does not occur directly, but *via* an intermolecular reaction with the C-terminus of another R1 subunit, where a CX_2C motif functions as an intermediate in reducing the active site disulphide bond (reviewed in [21]). Thioredoxin and glutaredoxin can then reduce the C-terminal disulphide bond. In contrast to Class I enzymes, Class II RNRs (NrdJ) function independently of oxygen and have single subunit which requires 5′-deoxyadenoysylcobalamin for radical generation. Class III enzymes (NrdD) are only active under anaerobic conditions, and use a stable glycine radical for catalysis which is generated with the aid of a second protein NrdG.

RNRs show a particularly elaborate mechanism of allosteric regulation which serves to regulate levels and relative amounts of dNTPs (reviewed in [17]). This involves binding of dATP or ATP to an activity site in the R1 subunit, which respectively inhibits or stimulates the enzyme. A second 'specificity' allosteric site affects the types of nucleotides reduced; thus binding of ATP or dATP stimulates the reduction of pyrimidine nucleotides, while TTP and dGTP stimulate GDP and ADP reduction respectively. The exact stoichiometry of the enzyme has been somewhat unclear, but recent findings suggest a $R1_6R2_2$ ring complex for the inactive and possibly also the active form ([22], reviewed in [23]).

Table 1
Changes in of dNTP levels in S phase or after DNA damage compared to G1 or G0 levels.

	S phase	DNA damage	Reference
S. cerevisiae	x3-6	x6-8	[2]
S. pombe	x2	x2	[14]
Mammalian	x18	<x2	[15,20]

3. Regulation of RNR levels during the cell cycle and after DNA damage

One conserved theme with eukaryotic RNR genes is transcriptional activation during S phase and after DNA damage (reviewed in [18]). In yeasts, two transcriptional pathways are involved, one of which is responsible for cell cycle dependent changes in transcription and another which is activated by DNA damage. *S. cerevisiae* has two R1 genes (*RNR1*, *RNR3*) and two R2 genes (*RNR2*, *RNR4*). *RNR1*/R1 is differentially regulated by the MBF transcription factor, which regulates many other G1/S genes, while *RNR2*/R2, *RNR3*/R1 and *RNR4*/R2 show little variation in expression during the cell cycle [24–29]. In response to DNA damage, the Dun1 kinase is activated by the Mec1-Rad53 pathway and phosphorylates Crt1, thus relieving repression of *RNR2-4* genes. Activation of *RNR1*/R1 upon DNA damage involves the HMG-transcription factor Ixr1, which binds to the *RNR1* promoter and also promotes transcription under basal conditions [30]. The TOR pathway has also been implicated in RNR activation after DNA damage, as inhibition of TORC1 with rapamycin interferes with activation of *RNR1*/R1 and *RNR3*/R1 expression after DNA damage, leading to increased sensitivity to DNA damaging agents [31]. The DNA-binding factor Rap1, which has multiple roles at telomeres, promoters and silencers, is also required for the activation of *RNR2-4* genes after DNA damage, thus changes in the level of Rap1 could potentially modulate RNR responses [32]. A recent study examined RNR mRNA and protein induction after DNA damage in single cells, and showed that elevation of both mRNA and protein was cell-cycle dependent, being striking in S/G2 cells but little affected in G1 cells [33].

In *S. pombe*, the story is similar in that the *cdc22*$^+$/R1 gene is cell cycle regulated by the MBF regulator of G1/S transcription [34]. The *suc22*$^+$/R2 gene generates a smaller, constitutively expressed transcript, and a larger one which is MBF-regulated and induced by DNA damage and heat-shock [35]. The Ino80 nucleosome-remodelling complex appears to be necessary for correct *cdc22*$^+$/R1 expression under basal conditions [36]. After DNA damage, the checkpoint kinase Cds1(Chk2) phosphorylates the Yox1 inhibitor of MBF, allowing reactivation of MBF and transcription of targets such as *cdc22*$^+$/*R1* [37]. For Suc22/R2, regulation of expression may also occur post-transcriptionally since a cytoplasmic poly(A)

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