Ribonucleotide Reductases

Ribonucleotide reductases [EC 1.17.4.] are a group of enzymes which critically contribute to the biosynthesis of deoxyribonucleotides (dNTPs), the monomeric precursors of DNA polymerization. By a radical reaction mechanism, they catalyze the reduction of the ribonucleotides, ADP, GDP, CDP, and UDP, mostly at the diphosphate level, to their deoxy counterparts dADP, dGDP, dCDP, and dUDP, respectively. Together with other enzymes, i.e. nucleoside and nucleotide kinases and phosphatases, deaminases, methylases and hydroxymethylases they are part of the biochemical pathway ultimately leading to dATP, dGTP, dCTP, and dTTP, the final substrates of DNA polymerases [31].

Of critical importance is not the amount of dNTPs, but are the relative concentrations of the four at the replication forks. Elongating and proofreading activities of DNA polymerases are affected by alterations of these concentrations, and biased dNTP concentrations are mutagenic. The enzymes in the deoxynucleotide biosynthetic pathway, especially ribonucleotide reductase which reduces all four ribonucleotide substrates, underlie extensive allosteric regulations with the effect to bring about a balanced and species-specific supply of dNTPs [18, 24]. Ribonucleotide reductases have a medium-complex subunit organization with an elaborate array of active and allosteric nucleotide binding sites. Most well-known is the \( \alpha_2 \beta_2 \) iron-dependent tyrosyl radical specimen of aerobic E. coli and eukaryotes [31]. Mutants of E.coli ribonucleotide reductase have been engineered which produce different deoxynucleotide ratios and act as mutator [1]. They induce base replacements in the direction of the nucleotides which they provide in excess.

There are different classes of ribonucleotide reductases in which the functionally important, protein side chain based radical is obtained by an upstream reaction sequence involving diverse metal cofactors [27, 35]. Radical acquisition can be either oxygen-dependent, oxygen-independent, or oxygen-sensitive, i.e. strictly anaerobic [39]. Reduction equivalents are provided by thioredoxins and glutaredoxins [19].

Deoxynucleotide synthesizing enzymes are moderately cell-cycle regulated. This can most significantly be observed in unicellular green algae grown in a 24 h light-dark-regime. In the dark, cells enter G0 phase, and ribonucleotide reductase activity vanishes [4, 14].

Viral Ribonucleotide Reductase Genes and Enzymes

Genes for ribonucleotide reductases are found in most viruses with genome sizes of 100 kbp and more. The first and best studied examples are in E.coli bacteriophage T4 [29, 39], Herpes viruses [12, 30], and the poxvirus, Vaccinia virus [34, 36]. In these cases, virus-coded ribonucleotide reductases have been expressed, separated from their host cell counterparts, and their enzyme properties have been characterized [2, 3, 17, 18, 22]. The allosteric properties, especially the relative yields of the four products
dADP, dGDP, dCDP, and dUDP, have been found to differ from those of the host enzymes in T-phage and Herpes viruses, but not in Vaccinia virus.

Significantly, only a few viruses like phage T4 encode a complete deoxynucleotide biosynthetic pathway [29]. It even possesses, like its host, an alternative anaerobic ribonucleotide reductase [39]. Most viruses do not play with a full deck [37], and some, e.g. the herpesvirus Human Cytomegalovirus, encode only one subunit of ribonucleotide reductase, which on its own is probably non-functional [8, 9].

Genes with sequence homology to ribonucleotide reductase continue to be found in baculoviruses [21, 38], African Swine Fever Virus [5], Chlorella Virus [25], Mimivirus [33], and, recently, in over 100 more bacteriophages [13], with cases again of lone subunits and with degenerating genes among them.

**Functions of Viral Ribonucleotide Reductases**

With accumulating DNA sequence data suggesting that viruses are rather gene shuttles than functional units and as it becomes unclear whether they are under selective pressure all the time [16] one is reluctant to ponder on possible functions of virus-specified genes. Viruses surely contain a core set of genes which are functional and indispensable for their maintenance and propagation, e.g. those for shell proteins. Small viruses like Simian virus 40 and adenoviruses further possess multifunctional gene products like their large T antigen or E1A which induce and recruit host cell functions needed for replication [11]. Large DNA viruses on top of this basic inventory encode functional homologues of cellular proteins. In Herpes and pox viruses, some of these are probably useful for evasion from the host immunosystem [6, 32].

Genes encoding enzymes of deoxyribonucleotide metabolism can in principle only be “functional”, in the sense of conferring independence from the host, if the pathway is complete. In most large viruses this is not the case. Especially thymidylate synthase which is indispensable for the formation of dTMP from dUMP is rarely encoded by viruses [37]. Viral ribonucleotide reductase genes are often obviously functionless, i.e. lone or degenerate [13].

When discovered, viral ribonucleotide reductases were regarded as possible therapeutic targets. Considerable effort went into elucidation of the significance of Herpesvirus ribonucleotide reductases [2, 10, 15] and their use as an Achilles heel in chemotherapy. All these attempts gave no useful results [23], obviously because virus-specified ribonucleotide reduction is not limiting in the infective cycle.

Deoxynucleotides are readily provided by the host cell dNTP-synthesizing machinery which is induced during infection (“S Phase-like environment” [7]).

If we lavish calculate dNTPs needed for replication of a virus with a large burst size and 90% waste DNA (i.e. not properly resolved and not packaged into virions), we arrive at 200 kbp x 1000 plaque forming units/cell x 10 which gives 2 x 10^9 base pairs. This number corresponds to an average eukaryotic genome size. So there is no demand for higher amounts of deoxynucleotides upon virus infection.

In conclusion virus – encoded ribonucleotide reductases rarely function as the sole and indispensable source of the deoxynucleotides needed for DNA replication. They merely “interfere with nucleic acid metabolism” [37].

**Possible Effects of Viral Ribonucleotide Reductases**

Nonetheless, virus – encoded ribonucleotide reductases are expressed during the infective cycle along with the host enzymes and will contribute to and alter dNTP pools according to their specific and sometimes different regulatory properties. The guanine + cytosine vs. adenine + thymine molar ratios of Herpesvirus
genomes encompass the whole viable range. Honess [20] suggested that this be due to a non selective mutation mechanism probably residing in their nucleotide metabolism. This could possibly be the allosteric properties of ribonucleotide reductase. For Herpes simplex virus 1 the low Michaelis constants of its ribonucleotide reductase for GDP and CDP [2] are in accord with the high (70%) guanine + cytosine content of its genome, and for other viruses this should be testable. For example two baculoviruses, Cydia pomonella granulovirus (CpGV), and Cryptophlebia leucotreta granulovirus (CrleGV), are closely related and highly homologous at the protein level but differ in the adenine + thymidine content of their DNA (55 vs 68%) [28]. This difference is mainly reflected in the wobble position of codons. CpGV encodes a ribonucleotide reductase, and CrleGV does not.

When the base composition of a viral genome is driven to an extreme where most further base replacements in the biased direction would result in non-functional proteins and thus less viable offspring, virus coded ribonucleotide reductases with mutator properties may exert a selective advantage. Further base replacements in the biased direction would be fatal, and base replacements against the biased direction are disfavoured, because these nucleotides are already at shortage. Thus, in a biotope with a given number and array of infectable host cells, a smaller number of still possible mutations can be probed more effectively for further development. There would be less competition with an otherwise vast number of neutral mutations.

Thus, forcing a GC/AT bias as extreme as possible is like erecting a wall. It would be like building a stable borderline along which the genome can evolve. The mutating force would be the altered deoxynucleotide concentrations brought about by a differently regulated ribonucleotide reductase and the selective force would be the maintenance or improvement of biological function of the proteins encoded by the GC/AT biased viral genome. Completely detached from their commonly assumed function, viral ribonucleotide reductases would then initially be non-selective Honessian mutators. Yet, as they drive them to their base composition extreme, they would gradually make their viruses quicker, more aggressive evolvers.

Acknowledgements

I wish to dedicate this article to the memory of Hartmut and Ursula Follmann.

References

1. Ahluwalia D, Bienstock RJ, Schaaper RM (2012) Novel mutator mutants of E. coli nrdAB ribonucleotide reductase: insight into allosteric regulation and control of mutation rates. DNA Repair 11: 480 - 487
2. Averett DR, Lubbers C, Elion GB, Spector T (1983) Ribonucleotide reductase induced by Herpes simplex type 1 virus. Characterization of a distinct enzyme. J Biol Chem 258: 9831 - 9838
3. Berglund O (1972) Ribonucleoside diphosphate reductase induced by bacteriophage T4. J Biol Chem 247: 7270 - 7281
4. Bornemann C, Drude K, Follmann H (1996) Deoxyribonucleotide synthesis in green algae. Cell cycle fluctuation of ribonucleotide reductase is only moderate in unicellular, exsymbiotic green algae, Clorella sp. pbi. J Plant Physiol 148: 657 - 661
5. Boursnell M, Shaw K, Yañez RJ, Viñuela E, Dixon L (1991) The sequences of the ribonucleotide reductase genes from African swine fever virus show considerable homology with those of the orthopoxvirus Vaccinia virus. Virology 184: 411 - 416
6. Bugert JJ, Darai G (2000) Poxvirus homologues of cellular genes. Virus Genes 21: 111-133
7. Castillo JP, Kowalik TF (2002) Human cytomegalovirus immediate early proteins and cell growth control. Gene 290: 19 - 34
8. Cerqueira NMFS, Pereira S, Fernandes PA, Ramos MJ (2005) Overview of ribonucleotide reductase inhibitors: an appealing target in anti-tumor therapy. Curr Med Chem 12: 1283 - 1294
9. Chee MS, Bankier AT, Beck S, Bohmi R, Brown CM, Cerny R, Hornsnell T, Hutchinson CA, Kouzarides T, Martignetti JA, Preddie E, Satchwell SC, Tomlinson P, Weston KM, Barrell BG (1990) Analysis of the protein-coding content of the sequence of human cytomegalovirus strain AD169. Curr Top Microbiol Immunol 154: 125 - 169
10. Conner J, Marsden H, Clements JB (1994) Ribonucleotide reductase of herpesviruses. Rev Med Virol 4: 25 - 34
11. DiMaio D, Coen DM (2001) Replication strategies of DNA viruses. In: Knipe DM, Howley PM (eds) Fundamental virology, 4th edn. Lippincott Williams & Wilkins, Philadelphia, pp 119 - 132
12. Dutia BM (1983) ribonucleotide reductase induced by Herpes simplex virus has a virus-specified constituent. J Gen Virol 64: 513 – 521
13. Dwivedi B, Xue B, Lundin D, Edwards RA, Breitbart M (2013) A bioinformatic analysis of ribonucleotide reductase genes in phage genomes and metagenomes. BMC Evolut Biol 13: 33
14. Feller W, Schimpff – Weiland G, Follmann H (1980) Deoxyribonucleotide biosynthesis in synchronous algae cells. Eur J Biochem 110: 85 - 92
15. Goldstein DJ, Weller SK (1988) Herpes simplex virus type 1-induced ribonucleotide reductase activity is dispensable for virus growth and DNA synthesis: isolation and characterization of an ICP6 lacZ insertion mutant. J Virol 62: 196 - 205; Goldstein DJ, Weller SK (1988) Factor(s) present in Herpes simplex virus type 1-infected cells can compensate for the loss of the large subunit of the viral ribonucleotide reductase: characterization of an ICP6 deletion mutant. Virology 166: 41 - 51
16. Goldenfeld N, Woese C (2007) Biology’s next revolution. Nature 445: 369
17. Hendricks SP, Mathews CK (1997) Regulation of T4 phage aerobic ribonucleotide reductase. J Biol Chem 272: 2861 – 2865
18. Hendricks SP, Mathews CK (1988) Allosteric regulation of Vaccinia virus ribonucleotide reductase, analyzed by simultaneous monitoring of its four activities. J Biol Chem 273; 29512 - 29518
19. Holmgren A (1989) Thioredoxin and glutaredoxin systems. J Biol Chem 264: 13963 - 13966
20. Honess RW (1984) Herpes simplex and “the herpes complex”: diverse observations and a unifying hypothesis. The eighth Fleming lecture. J Gen Virol 65: 2077 - 2107
21. Hughes AL, Friedman R (2003) Genome-wide survey of genes horizontally transferred from cellular organisms to baculoviruses. Mol Biol Evol 20: 979-987
22. Huszar D, Bacchetti S (1981) Purification and characterization of the ribonucleotide reductase induced by Herpes simplex infection of mammalian cells. J Virol 37: 580 - 588
23. Kleymanns G (2003) Novel agents and strategies to treat Herpes simplex virus infections. Expert Opin Investig Drugs 12: 165 - 183
24. Kunz BA, Kohalmi SE, Kunkel TA, Mathews CK, McIntosh Em, Reidy JA (1994) Deoxyribonucleoside triphosphate levels: a critical factor in the maintenance of genetic stability. Mutat Res 318: 1 - 64
25. Kutish GF, Li Y, Lu Z, Furuta M, Rock DL, van Etten JL (1996) Analysis of 76 kb of the Chlorella virus PBCV-1 330-kb genome: map positions 182 – 258. Virology 223, 303 - 317
26. (deleted)
27. Lammers M, Follmann H (1983) The ribonucleotide reductases: a unique group of metalloenzymes essential for cell proliferation. Structure and Bonding 54: 27 - 91
28. Lange M, Jehle JA (2003) The genome of the Crytophlebia leucotreta granulovirus. Virology 317: 220-236
29. Mathews CK, Kutter EM, Mosig G, Berget PB (1983) (eds) Bacteriophage T4. American Society for Microbiology, Washington, D.C.
30. McLauchlan J, Clements JB (1983) DNA sequence homology between two co-linear loci on the HSV genome which have different transforming abilities. EMBO J 2: 1953 - 1961
31. Nordlund P, Reichard P (2006) Ribonucleotide reductases. Annu Rev Biochem 75: 681 - 706
32. Raftery M, Müller A, Schönrich G (2000) Herpes virus homologues of cellular genes. Virus Genes 21: 65 - 75
33. Raoult D, Audic S, Robert C, Abergel C, Renesto P, Ogata H, La Scola B, Suzan M, Claverie JM (2004) The 1,2 megabase genome sequence of mimivirus. Science 306: 1344 - 1350
34. Slabaugh MB, Roseman N, Davies R, Mathews CK (1988) Vaccinia virus – encoded ribonucleotide reductase: sequence conservation of the gene for the small subunit and its amplification in hydroxyurea-resistant mutants. J Virol 62: 519 - 527
35. Stubbe JA (1990) Ribonucleotide reductases: amazing and confusing. J Biol Chem 265: 5329-5332
36. Tengelsen LA, Slabaugh MB, Bibler JK, Hruby DE (1988) Nucleotide sequence an molecular genetic analysis of the large subunit of ribonucleotide reductase encoded by Vaccinia virus. Virology 164: 121 - 131
37. Tidona CA, Darai G (2000) Iridovirus homologs of cellular genes – implications for the molecular evolution of large DNA viruses. Virus Genes 21: 77 – 81
38. Van Strien EA, Faktor O, Hu ZH, Zuidema D, Goldbach RW, Vlak JM (1997) Baculoviruses contain a gene for the large subunit of ribonucleotide reductase. J Gen Virol 78: 2365-2377
39. Young P, Öhman M, Xu MQ, Shub DA, Sjöberg BM (1994): Intron-containing T4 bacteriophage gene sunY encodes an anaerobic ribonucleotide reductase, J Biol Chem 269: 20229 - 20232