bustness" hypothesis (Preitner et al., 2002). A second possible function for the second feedback loop is to transduce signals from a variety of environmental inputs to the first feedback loop (Preitner et al., 2002). A third possibility is that the second feedback loop controls the expression of time-specific circadian outputs. Several DNA microarray studies have identified dozens of genes under clock control with distinct temporal profiles (for example, McDonald and Rosbash, 2001). The second feedback loop may even be important for turning on rhythmic gene expression in development. *Drosophila* genetics should continue to be important in determining the function of these interdependent feedback loops in circadian systems.

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Selected Reading

Allada, R., Emery, P., Takahashi, J.S., and Rosbash, M. (2001). Annu. Rev. Neurosci. *24*, 1091–1119.

Blau, J., and Young, M.W. (1999). Cell *99*, 661–671.

Cyran, S.A., Buchsbaum, A.M., Reddy, K.L., Lin, M.-C., Glossup, N.R.J., Hardin, P.E., Young, M.W., Storti, R.V., and Blau, J. (2003). Cell *112*, this issue, 329–341.

Glossop, N.R., Lyons, L.C., and Hardin, P.E. (1999). Science *286*, 766–768.

Glossop, N.R.J., Houl, J.H., Zheng, H., Ng, F.S., Dudek, S.M., and Hardin, P.E. (2003). Neuron *37*, 249–261.

Kim, E.Y., Bae, K., Ng, F.S., Glossop, N.R., Hardin, P.E., and Edery, I. (2002). Neuron *34*, 69–81.

McDonald, M.J., and Rosbash, M. (2001). Cell *107*, 567–578.

Mitsui, S., Yamaguchi, S., Matsuo, T., Ishida, Y., and Okamura, H. (2001). Genes Dev. *15*, 995–1006.

Preitner, N., Damiola, F., Lopez-Molina, L., Zakany, J., Duboule, D., Albrecht, U., and Schibler, U. (2002). Cell *110*, 251–260.

Shearman, L.P., Sriram, S., Weaver, D.R., Maywood, E.S., Chaves, I., Zheng, B., Kume, K., Lee, C.C., van der Horst, G.T., Hastings, M.H., and Reppert, S.M. (2000). Science *288*, 1013–1019.

Better Chemistry for Better Survival, through Regulation

In this issue of *Cell*, Chabes et al. (2003) report on new aspects of the regulation of yeast ribonucleotide reductase, the mechanism by which dNTP levels are increased following DNA damage, and the consequences of the metabolic changes.

The maintenance of a cell's genome is a biological imperative. When DNA is damaged in a eukaryotic cell, the cell cycle is arrested, and the enzymatic resources needed for DNA repair are transcriptionally induced or otherwise activated. The regulated genes include many encoding DNA repair enzymes, others involved in cell cycle control, and the subunits of ribonucleotide reductase. As reported by Chabes et al. in this issue of *Cell*, ribonucleotide reductase lies at the center of an elaborately regulated pathway that supplies not only the enzymes required for DNA repair, but also the metabolic precursors required by those enzymes.

Ribonucleotide reductase (RNR) has been surprising and delighting researchers for decades (Jordan and Reichard, 1998). In the absence of a reasonable abiotic pathway to deoxyribonucleotides, the enzyme has evolutionary importance as a prerequisite for the transition from RNA to DNA worlds. The reaction itself, the replacement of a carbon-linked hydroxyl group with hydrogen, had no precedent in synthetic organic chemistry when the enzyme was first discovered. RNR was also the first enzyme found to make use of free radical chemistry. Even the regulation of the enzyme provided some new lessons, as not only the activity is regulated, but also the specificity of the enzyme.

There are three well-characterized classes of ribonucleotide reductases, each utilizing a similar chemical strategy but exhibiting little sequence similarity and relying on different cofactors. Reichard (Jordan and Reichard, 1998) has argued that the class III enzymes, found in some anaerobically growing bacteria, are the closest to the RNR progenitor. Oxygen destroys the stable glycyl radical in class III enzymes. The appearance of oxygen in the atmosphere then led to the divergent evolution of class II enzymes, which are not affected by oxygen, and class I enzymes, which require oxygen. There are two variations of class I, called Ia and Ib. Similarities in reaction mechanism, regulation, and overall structure have helped bolster the common origin view (Jordan and Reichard, 1998; Stubbe, 2000). Many bacteria encode multiple ribonucleotide reductases, often from different classes. Eukaryotes generally possess class Ia enzymes, and yeast relies on a somewhat idiosyncratic version of the class Ia family (Chabes et al., 2000; Voegtli et al., 2001). Class Ia ribonucleotide reductases generally have an $\alpha_2\beta_2$ architecture. The active site, as well as all regulatory sites, is located in the large α subunit. Yeast has two genes encoding α subunits (*RNR1* and *RNR3*) and two encoding the smaller β subunits (*RNR2* and *RNR4*). All but *RNR3* appear to be essential. The small subunits may function as a heterodimer (Chabes et al., 2000; Voegtli et al., 2001).

The fundamental task of ribonucleotide reductases in cellular metabolism is to provide deoxynucleotides to support DNA synthesis. The same RNR active site accommodates all four ribonucleoside diphosphate substrates. However, the specificity at any given moment depends on the concentrations of different dNTPs and ATP, which all interact at the same allosteric binding site, called the specificity site. A complex regulatory scheme in which enzyme active site specificity depends on the nucleotide bound at the specificity site was laid out by Thelander and Reichard in 1979 (Thelander and Reichard, 1979). The scheme is designed to balance the concentrations of the various dNTPs as needed for replication. The same specificity modulation is observed with all classes of ribonucleotide reductases. In addition to this regulation of specificity, class Ia and class III enzymes have a second allosteric site, called the activity site. Binding of ATP or dATP to this site leads to enhancement or inhibition, respectively, of overall enzyme activity. In the yeast RNR, the inhibition by dATP is somewhat relaxed (observed at higher dATP concentra-

tions than seen with the mammalian enzymes), although still functionally important.

A consideration of DNA repair has added two chapters to the RNR regulation story, as reviewed by the authors in Chabes et al. (2003). First, DNA damage leads to an increase in transcription of the yeast *RNR* genes. Second, ribonucleotide reductase is inhibited by interaction with the protein Sml1. After DNA damage, the protein kinases Mec1 and Rad53 induce the transcription of the *RNR* genes and inactivate Sml1 (Zhao and Rothstein, 2002), increasing overall ribonucleotide reductase levels and activity. In Chabes et al. (2003), the Rothstein and Thelander groups build on their recent work and fill in this story. They show that DNA damage results in a substantial increase in dNTP levels in yeast, analyze the contributions of the various regulatory mechanisms to this increase, and show that the increase is important for cell survival following a DNA insult.

What Chabes et al. do particularly well is to open many potentially lucrative paths to further insight. The authors explore several types of DNA damage, but it is possible that different challenges might lead to different effects on dNTP levels. Does the cryptic *RNR3* gene product play any role in the increase in dNTP concentration (its levels are induced strongly by DNA damage, but deletion of the gene seems not to affect survival or overall RNR activity [Domkin et al., 2002])? And what makes use of the elevated dNTPs? The authors point out that some DNA polymerases specialized for translesion DNA synthesis (e.g., Pol η or ζ) have an elevated *K*m for inserting nucleotides opposite DNA lesions. Thus, translesion bypass of DNA damage may be enhanced by the elevated levels of dNTPs. The authors demonstrate that there is a price to pay for this in the form of increased mutation.

Are there other proteins with activities dependent on elevated dNTPs? In *E. coli,* the activity of the RecA protein is demonstrably enhanced if dATP is used in vitro in place of ATP (Menetski and Kowalczykowski, 1989; Shan et al., 1997). No in vivo function for this enhancement has ever been proposed, and mixtures of ATP and dATP designed to mimic the levels thought to exist in vivo do not produce the enhancement (Shan et al., 1997). One can imagine many other DNA repair functions in different organisms with similar activity alterations based on the nucleotide cofactor, and similar problems in determining their functional significance. Unfortunately, detailed concentration measurements of key cellular metabolites, obtained under a range of conditions, are not generally available for any model organism. The work reported by Chabes et al. recommends a more systematic examination of the broader metabolic consequences of DNA damage and the effects of those metabolic changes on DNA repair functions.

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Selected Reading

Chabes, A., Domkin, V., Larsson, G., Liu, A.M., Graslund, A., Wijmenga, S., and Thelander, L. (2000). Proc. Natl. Acad. Sci. USA *97*, 2474–2479.

Chabes, A., Georgieva, B., Domkin, V., Zhao, X., Rothstein, R., and Thelander, L. (2003). Cell *112*, this issue, 391–401.

Domkin, V., Thelander, L., and Chabes, A. (2002). J. Biol. Chem. *277*, 18574–18578.

Jordan, A., and Reichard, P. (1998). Annu. Rev. Biochem. *67*, 71–98.

Menetski, J.P., and Kowalczykowski, S.C. (1989). Biochemistry *28*, 5871–5881.

Shan, Q., Bork, J.M., Webb, B.L., Inman, R.B., and Cox, M.M. (1997). J. Mol. Biol. *265*, 519–540.

Stubbe, J. (2000). Curr. Opin. Struct. Biol. *10*, 731–736.

Thelander, L., and Reichard, P. (1979). Annu. Rev. Biochem. *48*, 133–158.

Voegtli, W.C., Ge, J., Perlstein, D.L., Stubbe, J., and Rosenzweig, A.C. (2001). Proc. Natl. Acad. Sci. USA *98*, 10073–10078.

Zhao, X., and Rothstein, R. (2002). Proc. Natl. Acad. Sci. USA *99*, 3746–3751.