higher atmosphere, where they "activate" into cloud droplets.

Pöschl *et al*. report that low aerosol concentrations resulted in cloud droplet number concentrations that were nearly independent of the updraft velocity of a convective cloud, and substantially higher supersaturation levels than found in polluted areas. This in turn meant that particles could activate to cloud droplets at a lower (smaller) critical diameter than in polluted areas. This modeling result is confirmed by measurements at the high-altitude (3580 m) station Jungfraujoch, Switzerland. There, the median of the activation diameter decreased from about 100 nm for particle concentrations (with diameter $D >$ 100 nm) greater than 100 cm^{-3} to about 65 nm for particle concentrations below 100 cm^{-3} (*7*). The effect of varying supersaturation is illustrated in the figure: At high supersaturation, a much higher fraction of the aerosol particles is activated to cloud droplets than at low supersaturation.

Pöschl *et al*. also report on ice nuclei (IN), particles that initiate ice formation at a temperature considerably above the freezing temperature of water (roughly –40°C). Supermicron-sized particles over the Amazon consisted mostly of primary biological aerosol particles that showed substantial IN activity. Precipitation occurs when these supermicron particles act as "giant" CCN (in warm rain) or IN (when ice formation is involved). The impact of aerosol particles on precipitation is different for warm and cold clouds. In warm clouds, increased CCN concentrations slow the conversion of cloud droplets into raindrops by nucleating larger concentrations of smaller drops, which are slower to coalesce into raindrops (*8*). In cold clouds, the situation is much more complex. The saturation vapor pressure is lower over ice than over liquid water; this transports water vapor from the cloud droplets to the ice crystals as soon as ice crystals form in liquid clouds (the so-called Wegener-Bergeron-Findeisen process) (*9*). This results in evaporation of the cloud droplets and a very low fraction of activated CCN (*10*). In these clouds, the radiative properties of the cloud are no longer influenced by the number of CCN but only by the properties of the IN and ice crystals. These processes are important for both the hydrological cycle and the radiative properties of clouds and clearly call for more research.

References

1. C. A. Pope, D. W. Dockery, *J. Air Waste Manage. Assoc.* **56**, 709 (2006).
2. IPCC, *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, S. Solomon *et al.,* Eds. (Cambridge Univ. Press, Cambridge, UK), www.ipcc.ch/ipccreports/ar4-wg1.htm (2007).
3. A. Clarke, V. Kapustin, *Science* **329**, 1488 (2010).
4. U. Pöschl *et al.*, *Science* **329**,1513 (2010).
5. S. Twomey, *J. Atmos. Sci.* **34**, 1149 (1977).
6. M. O. Andreae, *Science* **315**, 50 (2007).
7. S. Henning *et al*., *Tellus* **54B**, 82 (2002).
8. D. Rosenfeld *et al*., *Science* **321**, 1309 (2008).
9. W. Findeisen, *Meteorol. Z.* **55**, 121 (1938).
10. B. Verheggen *et al*., *J. Geophys. Res.* **112**, D23202 (2007).
11. E. Weingartner, S. Nyeki, U. Baltensperger, *J. Geophys. Res.* **104**, 26809 (1999).
12. G. McFiggans *et al*., *Atmos. Chem. Phys.* **6**, 2593 (2006).
13. U. Dusek *et al*., *Science* **312**, 1375 (2006).

10.1126/science.1192930

BIOCHEMISTRY

A Never-Ending Story

Revealing another way cells make DNA building blocks, this time with manganese.

Britt-Marie Sjöberg

More than 50 years ago, Reichard and colleagues elucidated how cells make their DNA building blocks—the deoxyribonucleotides or dNTPs (*1*). They found that the enzyme ribonucleotide reductase (RNR) converts ribonucleotides (RNA building blocks) to corresponding dNTPs. One would expect that such a central pathway for all living cells would be meticulously mapped by now. Yes—and no. Researchers have described several classes and subclasses of RNRs (see the figure) that appear to have the same evolutionary origin (*2*–*5*), but involve different chemical cofactors, and so enable cells to construct dNTPs under different environmental conditions. Whenever the field seems settled, however, fascinating new aspects appear (*1*, *2*). On page 1526 of this issue, Boal *et al*. (*3*) report crystal structures of RNR complexes from the bacterium *Escherichia coli* that, together with earlier studies, confirm and neatly illuminate yet another way cells can construct dNTPs, this time with the help of manganese (Mn).

Department of Molecular Biology and Functional Genomics, Stockholm University, SE-10691 Stockholm, Sweden
E-mail: britt-marie.sjoberg@molbio.su.se

Early researchers initially identified two classes of RNR: class I, which is characterized by a nonheme diiron center and a protein-derived tyrosyl radical (Fe^{III}_2-Tyr•); and class II, which involve the vitamin B_{12} coenzyme 5′-deoxyadenosylcobalamin (AdoCbl). Both of these RNR classes operate when oxygen is present (aerobic conditions). Later, investigators identified an anaerobic class III RNR, which involves a glycyl radical cofactor (Gly•) generated by an iron-sulfur cluster that cleaves S-adenosylmethionine (AdoMet). Despite structural differences and the involvement of different cofactors (see the figure), all RNR classes have a common origin and generate a transient thiyl radical (Cys•) in the active site.

In 1988, investigators isolated a Mn–dependent RNR from *Corynebacterium ammoniagenes* (*6*). It was not recognized as its own class, however; gene sequencing classified it as part of RNR class Ib, a subclass of class I. In addition, the role of Mn was unclear, since the new RNR was active in vitro with an iron cofactor (Fe^{III}_2-Tyr•) (*7*). Now, Boal *et al*., together with Cox *et al*. (*8*), highlight the importance of the Mn form of the class Ib RNR in *C. ammoniagenes* and *E. coli*. The work also highlights the role of the protein NrdI, a flavodoxin that is a crucial player in the formation of Mn-RNR. NrdI is encoded in the same operon as NrdE and NrdF, the two components of the known class Ib RNR. NrdI is essential for the formation of Tyr• in Mn-NrdF (*9*).

The NrdIs are unusual flavodoxins. They are smaller than classical flavodoxins, with one notable variation in the loop that interacts with the flavin mononucleotide (FMN) (*3*, *10*). Recent structures for NrdIs from *Bacillus anthracis* (*10*), *Bacillus cereus* (*11*), and *E. coli* (*3*) cover the three redox forms of FMN (oxidized, semiquinone, and hydroquinone). Whereas the two redox potentials of classical flavodoxin differ by more than 100 mV (*12*), *E. coli* NrdI maintains two almost identical redox potentials (*9*). The semiquinone form is thus transient in *E. coli* NrdI, which probably functions as a two-electron donor. Several NrdIs differ markedly from classical flavodoxin in their isoelectric points (pIs). Whereas flavodoxins have very similar pIs (4.5 ± 0.6), those of *E. coli* and *C. ammoniagenes* NrdIs are much higher, and *B. cereus* and *B. anthracis* NrdIs have pIs like those of flavodoxins (*11*). Both *B. cereus* and *B. anthracis* NrdIs

have been crystallized in semiquinone form (*10*, *11*) and may be one-electron donors.

The studies on class Ib Mn-RNRs from *E. coli* by Boal *et al*. (*3*) and from *C. ammoniagenes* by Cox *et al*. (*8*) fit together like hand in glove. The high-resolution structures of *E. coli* Mn-NrdF/NrdI complexes highlight a specific channel connecting the FMN cofactor in NrdI to the Mn^{II}_2 site in NrdF. This perfect match between the two proteins is conceivably set up to transfer oxidant to the metal site during activation of NrdF. In previous studies, *C. ammoniagenes* class Ib RNR had been expressed in *E. coli*, but Cox *et al*. (*8*) chose the safe road and expressed high levels of *C. ammoniagenes* NrdF in its homologous background. The intrinsic NrdI probably works catalytically in this expression system and forms enzymatically active Mn-NrdF with Tyr•. Their high-resolution structure shows two Mn ions bridged by one oxo/hydroxo ligand and one carboxylate. Using advanced spectroscopic methods, the authors elegantly reason that the manganese site is Mn^{III}_2 and observe, with electron paramagnetic resonance, a deprotonated weakly H-bonded Tyr• cofactor.

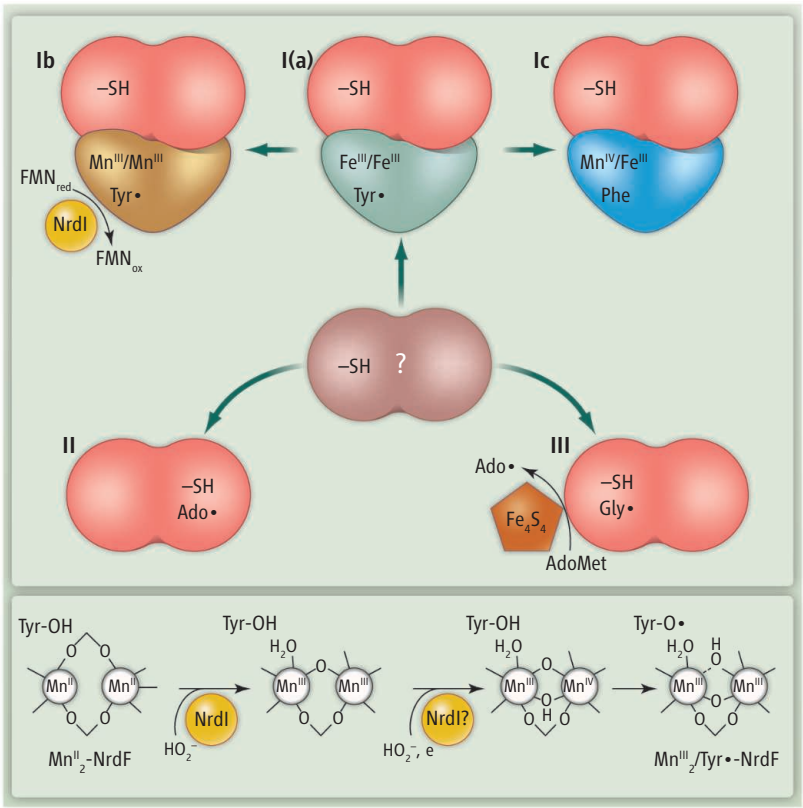
Just as there are acidic (*B. anthracis* and *B. cereus*) and basic (*E. coli* and *C. ammoniagenes*) NrdIs, the corresponding NrdFs group into different clades in a phylogenetic tree (*13*), with 67% identity between *E. coli* and *C. ammoniagenes* NrdFs and only ~40% identities between the *B. cereus* group NrdFs and the other two. In contrast to the Mn^{III}_2 site in *C. ammoniagenes*, the Mn^{II}_2 site in *E. coli* is bridged by three carboxylates (*3*). This metal ligation is unique to *E. coli* Mn-NrdF and differs from *C. ammoniagenes* Mn^{II}_2-NrdF with two bridging carboxylates (*14*).

Failure to oxidize isolated *E. coli* Mn^{II}_2-NrdF with small-molecule oxidants led investigators to propose that reduced NrdI reacts with O_2 to produce peroxide (HO_2^- or H_2O_2) (*9*). The channel in the Mn^{II}_2-NrdF/NrdI complex is wide enough to transfer a peroide species and is lined by hydrophilic residues and backbone atoms favoring the peroxide anion (*3*). Models suggest that density observed in one of the crystallized complexes is a peroxide species. Binding and heterolytic cleavage of the peroxide would generate a μ-oxo-bridged Mn^{III}_2 site (*9*). A second peroxide would generate Mn^{III}/Mn^{IV} mixed-valent NrdF with the potential to form the enzymatically active Mn^{III}_2-Tyr•. The figure shows a simplified reaction scheme for the *C. ammoniagenes* NrdF-NrdI couple (*8*).

Although the class Ib Mn-RNRs are now firmly established, several open questions remain. Are class Ib RNRs essential? *Streptococcus pyogenes* NrdI is essential for NrdEF function (*15*), *B. subtilis* class Ib RNR is essential both for aerobic and anaerobic growth (*16*), and the requirement of *C. ammoniagenes* and *C. glutamicum* for manganese is related to its class Ib RNR functionality (*8*). In contrast, *E. coli* uses class Ia RNR and not class Ib RNR for its aerobic growth, but oxidative stress and severe iron limitation may promote use of class Ib RNR and Mn-dependent growth (*9*).

Are class Ib RNRs active with either metal (cambialistic)? *C. ammoniagenes* and *E. coli* NrdFs can be reactivated in vitro with ferrous iron and O_2 to the classical Fe^{III}_2-Tyr• cofactor normally found in class Ia RNRs, and the Fe-NrdFs are enzymatically active. Are they cambialistic in vivo? The requirement of *C. ammoniagenes* for manganese argues against this idea, and it would be interesting to study how the NrdF-NrdI system may differentiate between Fe and Mn ions. In the presence of NrdI, Mn-NrdF has higher enzyme activity compared to Fe-NrdF (*9*).

Characterization of class Ib Mn-RNRs has been a long, meandering path spanning 30 years. It is now crowned by beautiful three-dimensional structures of a Mn^{III}_2 form of NrdF (*8*), as well as complexes of Mn^{II}_2-NrdF/NrdI (*3*), and these results add up to much more than their sum.



A dimanganese RNR subclass. Three major classes of RNR (I to III) have a common origin in the substrate-binding components (red) where the enzyme reaction occurs (**top**). Class Ib and Ic are subclasses of class I (often called class Ia). Canonical class I has a diiron/tyrosyl radical cofactor (green), subclass Ib has a dimanganese/tyrosyl radical cofactor (brown), and subclass Ic a manganese/iron mixed-valent cofactor (blue). The flavodoxin NrdI (yellow) is essential for generation of the tyrosyl radical in dimanganese class Ib. In a simplified reaction mechanism for formation of the dimanganese/tyrosyl radical cofactor (**bottom**), the FMN in NrdI forms peroxide via reduction of dioxygen.

References

1. P. Reichard, *Biochem. Biophys. Res. Commun.* **396**, 19 (2010).
2. P. Nordlund, P. Reichard, *Annu. Rev. Biochem.* **75**, 681 (2006).
3. A. K. Boal, J. A. Cotruvo Jr., J. Stubbe, A. C. Rosenzweig, *Science* **329**, 1526 (2010); published online 5 August 2010 (10.1126/science.1190187).
4. D. T. Logan, J. Andersson, B.-M. Sjöberg, P. Nordlund, *Science* **283**, 1499 (1999).
5. M. D. Sintchak, G. Arjara, B. A. Kellogg, J. Stubbe, C. L. Drennan, *Nat. Struct. Biol.* **9**, 293 (2002).
6. A. Willing, H. Follmann, G. Auling, *Eur. J. Biochem.* **170**, 603 (1988).
7. F. Fieschi *et al*., *J. Biol. Chem.* **273**, 4329 (1998).
8. N. Cox *et al*., *J. Am. Chem. Soc.* **132**, 11197 (2010).
9. J. A. Cotruvo Jr., J. Stubbe, *Biochemistry* **49**, 1297 (2010).
10. R. Johansson *et al*., *FEBS J.* **277**, (2010).
11. A. K. Røhr, H. P. Hersleth, K. K. Andersson, *Angew. Chem. Int. Ed. Engl.* **49**, 2324 (2010).
12. M. L. Ludwig *et al*., *Biochemistry* **36**, 1259 (1997).
13. D. Lundin, E. Torrents, A. M. Poole, B. M. Sjöberg, *BMC Genomics* **10**, 589 (2009).
14. M. Högbom, Y. Huque, B.-M. Sjöberg, P. Nordlund, *Biochemistry* **41**, 1381 (2002).
15. I. Roca, E. Torrents, M. Sahlin, I. Gibert, B.-M. Sjöberg, *J. Bacteriol.* **190**, 4849 (2008).
16. E. Härtig, A. Hartmann, M. Schätzle, A. M. Albertini, D. Jahn, *Appl. Environ. Microbiol.* **72**, 5260 (2006).

10.1126/science.1196347

CREDIT: P. HUEY/*SCIENCE*