MOLECULAR BIOLOGY

Dicer's Cut and Switch

An enzyme that cleaves RNA is converted to a DNA-cleaving enzyme during programmed cell death in *Caenorhabditis elegans*.

Qinghua Liu and Zain Paroo

Apoptosis is an essential developmental process in which a highly concerted series of biochemical events directs cell disassembly and death. Degradation of chromosomal DNA is a defining characteristic of apoptosis, involving distinct deoxyribonucleases that initiate DNA fragmentation (initiator DNases) and metabolize residual DNA (progressive DNases). On page 327 of this issue, Nakagawa *et al*. (*1*) report an unexpected role in apoptotic DNA degradation for an enzyme that was previously known for its role in RNA interference (RNAi).

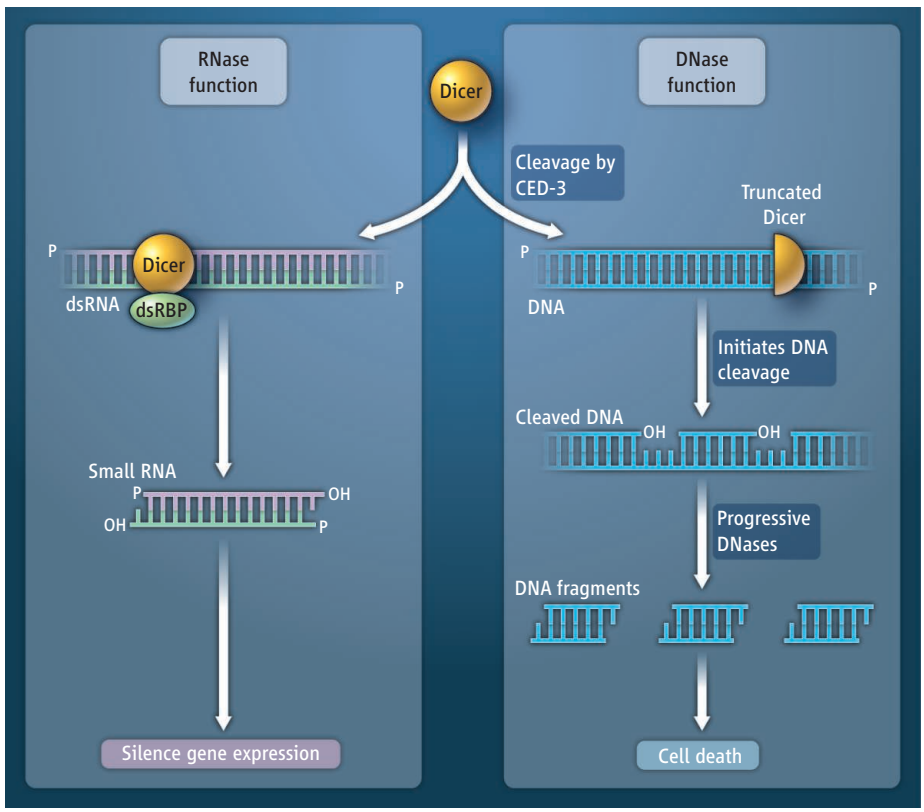
The first advance in understanding the mechanistic basis of apoptosis was the identification of *ced-3*, the gene that encodes a cysteine protease (caspase) in the nematode *Caenorhabditis elegans* (*2*, *3*). CED-3 and other caspases function in a cascade to activate a spectrum of effectors for cellular dissolution. Although progressive DNases are common to nematodes and mammals, initiators of DNA cleavage in *C. elegans* have been elusive. In mammals, DNA fragmentation factor (DFF40) [also known as caspase-activated deoxyribonuclease (CAD)] is sequestered in a complex with DFF45 [also called inhibitor of CAD (ICAD)] (*4*, *5*). Upon induction of apoptosis, activated caspases cleave the inhibitory DFF45 subunit, thereby freeing the DFF40 subunit to initiate chromosome cleavage, generating 3′ hydroxyl DNA breaks.

To identify the functional equivalent of mammalian DFF in *C. elegans*, Nakagawa *et al*. conducted a targeted RNAi screen. Candidate initiator DNases were selected through informatics searches and not limited solely to annotated DNases. Surprisingly, a gene (*dcr-1*) encoding a ribonuclease called Dicer was identified as a weak hit in the screen. Dicers are highly conserved RNase III enzymes that function in tandem with double-stranded RNA binding proteins (dsRBPs) in processing dsRNA to small regulatory RNA (*6*, *7*) (see the figure). These small RNAs effect RNAi by programming Argonaute proteins to guide sequence-specific silencing of messenger RNA (*8*).

Recognizing that the targeting of *dcr-1* by RNAi was achieved with low efficiency, Nakagawa *et al*. confirmed the initial finding by examining two different nematode strains lacking the *dcr-1* gene, both of which exhibited a stronger apoptotic phenotype. Historically, loss-of-function experiments that involve RNAi components have been used to infer functional roles for small RNAs (*9*). Given the wide spectrum of biological processes governed by such regulatory RNAs, it seemed plausible that small RNAs might be involved in apoptotic DNA degradation. However, animals defective for other RNAi components did not produce the same phenotype as that of the *dcr-1* mutants, indicating an RNAi-independent role for Dcr-1 in apoptotic DNA fragmentation.

Nakagawa *et al*. postulated that Dcr-1 might be a direct target of the CED-3 caspase because the lack of functional Dcr-1 reduced the number of cell deaths induced by constitutively activated CED-3. Indeed, recombinant CED-3 cleaved Dcr-1 in its RNase IIIa domain, producing a truncated Dcr-1 consisting of only an RNase IIIb domain and a dsRNA binding domain. Because both RNase III domains are required for dsRNA processing (*10*–*12*), this cleavage abolished RNase activity. Remarkably, truncated Dcr-1, but not full-length Dcr-1, exhibited DNase activity, generating 3′ hydroxyl DNA breaks much like initiator DNases in mammalian cells. Moreover, mutation of catalytic RNase residues in full-length Dcr-1 abolished both RNA and DNA cleavage activity. These findings indicate that CED-3 switches Dcr-1 from an RNase to a DNase.

Through a series of elegant genetic experiments, Nakagawa *et al*. further demonstrate that cleavage of Dcr-1 by CED-3 is required for promoting apoptosis. In addition to deficiencies in apoptosis, *dcr-1* mutants exhibit aberrant vulvar anatomy due to defective production of microRNA. In *dcr-1* mutants,



RNA and DNA substrate crossover. The RNase III Dicer functions with a double-stranded RNA binding protein (dsRBP) in processing long double-stranded RNA (dsRNA) to small RNAs. In *C. elegans*, the CED-3 protease cleaves Dicer (or a Dicer-dsRBP complex), which inactivates its RNase function. The truncated Dicer product gains DNase function and initiates breaks on chromosomal DNA. Progressive DNases further metabolize DNA fragments to complete apoptotic DNA degradation.

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expression of a *dcr-1* transgene that lacks a cleavage site for CED-3 rescued the developmental (vulva) defect, but not the apoptotic phenotype. Conversely, transgenic expression of the truncated form of Dcr-1 in *dcr-1* mutants rescued apoptosis but not the vulvar phenotype.

This highly unexpected role for Dcr-1 in early apoptotic DNA degradation has numerous important implications. Caspase-mediated activation of DNA degradation now appears conserved from nematodes to mammals. Full-length Dcr-1 (and/or Dcr-1 in complex with a dsRBP) maintains an inactive DNase in a manner similar to the DFF40-DFF45 complex in mammals. Cleavage of Dcr-1 by CED-3 activates a functional DNase that generates 3′ hydroxyl DNA breaks, which are subsequently resolved by progressive DNases. Conservation of this enzymatic switch may mean that Dicer also serves as an apoptotic DNase in higher organisms. The DNase activity of Dicer may also govern other aspects of chromosome dynamics. Thus, it may be prudent to consider RNAi-independent mechanisms underlying the phenotypes of *dicer* mutants. Perhaps most exciting will be determining the extent to which regulators of nucleic acid function exhibit substrate crossover, and the mechanisms through which this switching may occur.

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CHEMISTRY

In Pursuit of Water Oxidation Catalysts for Solar Fuel Production

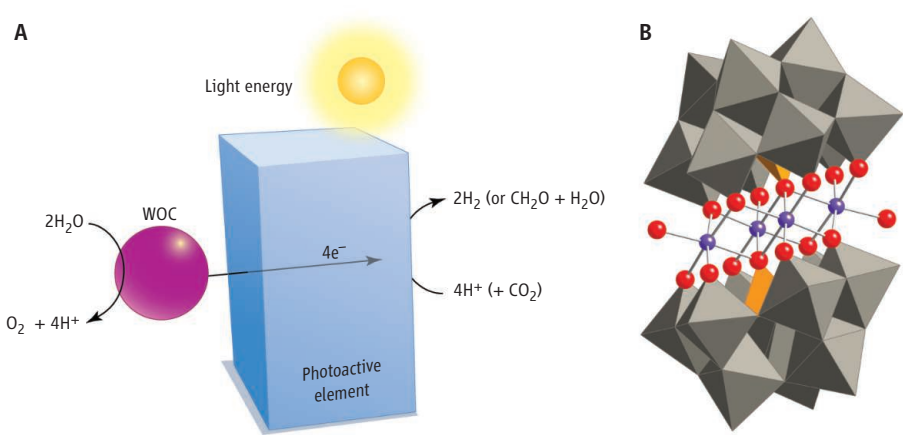
A durable molecular catalyst for water oxidation has been made from readily available and relatively inexpensive materials.

James K. Hurst

Roughly three-fourths of the power generated globally comes from burning fossil fuels. For solar energy to compete directly as a replacement, technologies are needed to capture this energy in a chemical form—as a fuel—so it can be used when sunlight is not available. One bottleneck in the development of practical solar fuels is the water oxidation reaction. Water is the only potential source of electrons capable of reducing protons to H_2 or CO_2 to carbonaceous fuels on a global scale. Thus, while there may be many options for reduction catalysts, redox cycling inevitably requires the coupling of reduction reactions to water oxidation (see the figure, panel A). On page 342 of this issue, Yin *et al.* (*1*) report on a water-soluble water oxidation catalyst that has a reaction center containing four cobalt (Co) atoms. Its surrounding ligands are not organic groups but are polyoxotungstate ($PW_9O_{34}^{9-}$) anions that resemble the solid oxide supports of heterogeneous catalysts. This catalyst weds the best features of extant heterogeneous and homogeneous catalysts while remedying many of their respective disadvantages.

The task of developing a catalyst that meets the diverse criteria for practical applications is daunting. The half-reaction comprising water oxidation, $2H_2O \rightarrow O_2 + 4H^+ + 4e^-$, is a chemist's nightmare, in that it involves multiple bond rearrangements between two molecules that must be carefully synchronized with the removal of protons and electrons if the formation of rate-retarding, high-energy intermediates is to be avoided. Moreover, to be of use in large-scale applications, the catalysts must stay active for extended periods of time or be easily regenerated, and must be made from readily available and inexpensive materials.

Both homogeneous and heterogeneous catalysts have advantages and drawbacks for water oxidation. Homogeneous catalysts permit maximal dispersion of catalytic centers (and hence maximal reactivity), and in most cases, many well-characterized variants can be synthesized with different ligand environments, All of these features foster performance optimization. However, currently available catalysts tend to rapidly lose their catalytic capabilities because they contain heterocyclic organic ligands that easily degrade (*2*–*4*) in the hostile environments required to oxidize water. These catalysts are



Artificial photosynthesis. (**A**) A water oxidation catalyst (WOC) transfers electrons from water to photoactive assemblies that promote photoinitiated charge separation for reduction of protons to hydrogen or carbon dioxide to organic compounds. In this generic scheme, the photoactive device comprising a photosensitizer and reduction catalyst might be assembled on an electrode, suspensions of small particles, or conducting organic or inorganic membranes capable of directing charge transport. Formaldehyde is shown as one of several possible CO_2 reduction products. (**B**) A schematic drawing of the water oxidation catalyst reported by Yin *et al.* depicts the Co_4O_{16} core with cobalt atoms in purple and oxygen atoms in red. The WO_6 units are depicted as gray octahedra, and phosphate groups are shown as orange tetrahedra.

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