removing the abundant (micromolar) cysteine seems an impervious task for the relatively few Tregs. The following mechanisms are more likely: preventing cystine uptake or GSH release and hydrolysis by DCs, or causing cysteine oxidation (Fig. 1). A mechanistic comprehension of the phenomena described remains a crucial task, as it could identify new targets to modulate DC and/or Treg activity.

Another open question is whether and how the changes in the extracellular redox state are restricted in the areas of DC–T cell interaction. If this were not the case, the nutrient-rich and reducing halo surrounding DCs could activate bystander cells, promoting unwanted responses. An exciting possibility is the polarized secretion of thiol-active enzymes, restricted to the immune synopsis, as described for IL-1β (ref. 13).

Skeptics could argue that the systemic control mechanisms would rapidly counteract these intercellular redox-based dialogs. However, in the constrained synapse, cysteine, other nonprotein thiols and redox enzymes could reach high concentrations. Also, in other tissues formed by cells tightly bound to each other, redox waves could be transmitted and could serve regulatory roles. Nonetheless, the skeptic point of view is reasonable: perhaps it is not surprising that such circuits have been described in inflammation and immunity, where signals ought to be clear and perceptible, but brief and short-range.

The identification of the redox-sensitive targets in Teffs and DCs, and in other systems where the release of cysteine/GSH serves a regulatory role, is clearly a priority. Likewise, the development of dyes reporting on intercellular redox changes is needed in order to fully appreciate the physiopathological implications of these modifications.


**DNA-catalyzed hydrolysis of DNA phosphodiesterse**

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Hydrolysis of the DNA backbone is an important reaction in biology and in the laboratory manipulation of genetic material. While many enzymes catalyze the sequence-selective hydrolysis of DNA, it has been difficult to design nonprotein catalysts for this purpose. In this issue1, Chandra et al. describe in vitro selection experiments that identified DNA catalysts that effectively carry out sequence-specific hydrolysis of DNA phosphodiesterse.

The uncatalyzed hydrolysis of DNA phosphodiesterse is thermodynamically favorable (∆G°′ = −5.3 kcal mol−1) (ref. 2) but kinetically very slow (Fig. 1a). The negative charge on the phosphodiester group protects it from hydrolysis, and oxidative cleavage of DNA1. In the experiment, Watson-Crick base pairing was used to “clamp” a peptide substrate segment across from a 40-nucleotide catalytic domain. These sequences were amplified and re-ligated to a substrate strand, and the product was identified in this study cleave DNA phosphodiesterse.

More hydrolytically labile. Indeed, an early report that ascribed thermal cleavage of DNA to phosphodiester hydrolysis may have inadvertently measured the rate of strand cleavage stemming from hydrolysis of the glycosidic bonds that hold the nucleobases to the sugar-phosphate backbone4. More recently, scientists have used cleverly designed non-nucleic acid model compounds to estimate the hydrolytic lability of DNA phosphodiester links5. The best current estimate for the half-life of hydrolytic cleavage of the DNA backbone rests at about 30,000,000 years5. At this rate, only about two phosphodiesterse within the 3 billion base pairs of DNA in a human cell are expected to undergo spontaneous hydrolysis per week. By way of comparison, it is estimated that 10,000 nucleotidc bonds undergo spontaneous hydrolysis per cell per day6.

Deoxyribozymes—short segments of DNA capable of catalyzing chemical reactions—have been developed for a number of reactions, including the hydrolytic cleavage of RNA phosphodiesterse, hydrolysis of the glycosidic bonds holding guanine residues to the DNA backbone7 and oxidative cleavage of DNA8. In their current work1, Chandra et al. initially set out to develop a deoxyribozyme catalyst that cleaves an amide bond in a peptide. This is a challenging reaction due to the fact that the inherent hydrolytic lability of amides is about 10-fold lower than that of RNA phosphodiesterse9. In the experiment, Watson-Crick base pairing was used to “clamp” a peptide substrate segment across from a 40-nucleotide stretch of randomized DNA sequence (Fig. 1b). In this manner, a vast number of potential DNA catalysts were rapidly screened for amidase activity. Polycrylamide gel electrophoresis was used to isolate the ‘winning’ sequences that caused strand cleavage in the region opposing the 40-nucleotide catalytic domain. These sequences were amplified and re-ligated to a substrate strand, and the process was repeated. After 10 rounds of this selection process designed to enrich for sequences capable of cleaving the substrate strand, the pool of winning sequences was able to cause 16% cleavage of the substrate strand over the course of a 2-h incubation (37 °C, pH 7.4) in a buffer containing MgCl2 (40 mM), MnCl2 (20 mM), ZnCl2 (1 mM) and NaCl (150 mM). From this collection, nine unique deoxyribozymes were identified and four were characterized in detail.

Surprisingly, all of the deoxyribozymes identified in this study cleave DNA phosphodiesterse...
Agents capable of sequence-selective DNA hydrolysis are important tools in molecular biology and biotechnology. In particular, the single-stranded nuclease deoxyribozymes described here may provide an excellent starting point for the development of "restriction deoxyribozymes" that sequence-selectively cleave duplex DNA. Metal ions are common cofactors in the enzymatic hydrolysis of phosphates and phosphodiesters, with the potential to (i) deliver metal-bound hydroxides and (ii) serve as Lewis acids. It may be of interest to determine the molecular details regarding how these DNA catalysts put Zn$^{2+}$ and Mn$^{2+}$ ions to work in DNA hydrolysis. It will also be valuable to learn whether various DNA functional groups directly play roles in catalysis. Regardless of the exact mechanism, this work expands the repertoire of deoxyribozymes to one of the most challenging chemical reactions in biology. This is fundamentally interesting and also of potential practical value.

**Figure 1** Deoxyribozyme-catalyzed cleavage of DNA. (a) The chemical reaction carried out by the deoxyribozymes. (b) Schematic design of the deoxyribozyme-substrate complexes.