these will surely vary in different places and at different times, perhaps substantially. For example, although tourism is important to many coastal economies along the west coast of Canada and the United States, this isn't so everywhere, nor is it likely that all local communities wish it to be. The ledger of net monetary benefit from sea otters would not switch to a net cost with the removal of tourism, but it would be greatly reduced, given the factors that Gregr *et al.* considered. The important point is that quantifying indirect effects broadens the base of stakeholders in natural resource management by identifying interests that could otherwise be overlooked or dismissed as trivial. Such an expanded perspective alerts the public that they may indeed have a stake in decisions made by natural resource agencies, and it directs policymakers and managers to constituencies they may not have previously consulted.

There is also the larger question of how the monetary costs and benefits of predators affect other ecosystems. This is an important future arena for science and policy. One such scenario involves the gray wolf, which has been the focus of similar controversy and societal discord. The ecological influences of wolves in North American boreal forests are broadly similar to those of sea otters and kelp forests (*5*). One especially topical segment of the web of interactions that emerges from wolves as predators is that of disease. The removal of wolves from the midwestern and eastern United States was likely instrumental in the spread of coyotes into these regions, which in turn depleted fox populations. This led to an increase in small-mammal populations, an associated increase in tick abundance, and the rise of Lyme disease risk (*6*). Such a possibility creates an entirely new dimension to the costs and benefits of living with wolves. The analysis by Gregr *et al.* should spark a new era of ecological-economic research that can be used by natural resource policy-makers and managers to make and defend more rational, equitable, and far-sighted decisions affecting predators. ■

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MOLECULAR BIOLOGY

CRISPR at lightning speeds

Breaking the genome with the flick of a (light) switch

By **Darpan Medhi** *and* **Maria Jasin**

Fundamental studies since the mid-1990s demonstrated the power of targeting DNA double-strand breaks (DSBs). This work uncovered a plethora of DNA repair pathways and formed the basis for the revolution in genome modification (*1*). That revolution in its most powerful manifestation came about with the discovery of RNA-directed DNA-cleaving enzymes in CRISPR systems, allowing the simple rules of base-pairing to guide targeted DNA breakage. Yet the CRISPR-associated 9 (Cas9) endonuclease that induces a DSB at target sites (determined by guide RNAs) in gene editing has been delivered as a relatively blunt instrument, with little control over its activity. On page 1265 of this issue, Liu *et al.* (*2*) developed Cas9 into a precision instrument that is both temporally and spatially controlled. It should now be possible to interrogate the cellular response to DSBs in real time, which will allow greater understanding of how cells maintain genome integrity when faced with these potentially catastrophic lesions.

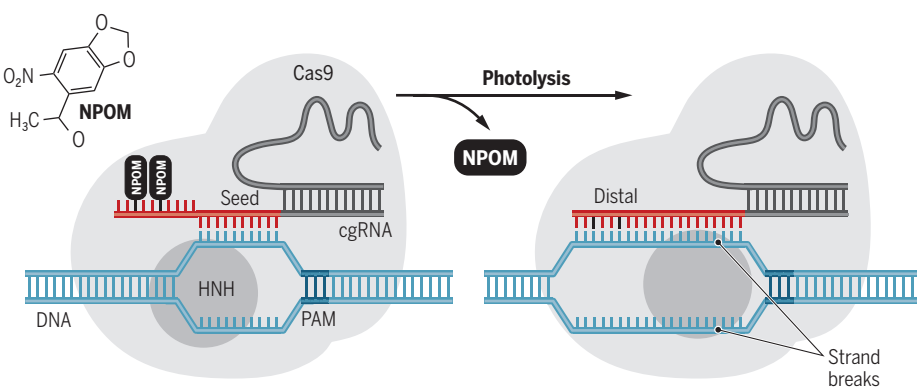
Gene-editing nucleases have historically been expressed in cells from plasmid vectors that rely on several steps (cellular uptake, transcription, and translation) before cleavage can occur. Once expressed, the nucleases may be present for days, or even permanently, if viral transduction is used. With the advent of CRISPR, direct delivery of active but relatively short-lived nucleases, in the form of Cas9–guide RNA ribonucleoproteins (RNPs) (*3*), has been feasible. However, even with RNP delivery, cleavage of genomic sites is not immediate and may not be synchronous (*3*), hampering the study of DNA breakage and repair in real time.

To overcome these limitations, Liu *et al.* developed very fast CRISPR (vfCRISPR) to enable precise control of Cas9 activity using light. Prebound to its target in genomic DNA through a modified guide RNA, Cas9 is activated to cleave DNA nearly instantaneously by shining light at wavelengths that are not phototoxic to cells (365 or 405 nm). The system uses standard CRISPR features, including protospacer adjacent motif (PAM) recognition by Cas9 and guide RNA binding close to the PAM (see the figure). However, distal guide RNA binding is blocked as a result of steric hindrance by modified deoxyribonucleotides (*4*) incorporated into the guide RNA. This "caged" guide RNA (cgRNA) effectively prevents complete R-loop (DNA-RNA hybrid) formation of the cgRNA with the target DNA (*2*), which is necessary to activate

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Photoactivation of CRISPR

After protospacer adjacent motif (PAM) recognition by CRISPR-associated 9 (Cas9), the caged guide RNA (cgRNA) forms base pairs with the target DNA at the PAM-proximal "seed" sequences. However, distal cgRNA sequences are prevented from binding by steric hindrance from 6-nitropiperonyloxymethyl (NPOM) thymidine. Photolysis of NPOM (365 or 405 nm) allows distal base pairing, provoking a conformational change of the Cas9 HNH endonuclease domain. This activates DNA cleavage by both nuclease domains.



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the Cas9 nuclease (*5*, *6*). Because the modified residues are photolabile, unmasking occurs rapidly upon light exposure to allow full DNA base pairing.

Light has long been recognized as a desirable agent for controlling all sorts of biological processes because it is noninvasive and provides exquisite spatial and temporal control (*7*, *8*). Methods of photoactivatable Cas9 cleavage were previously reported by other groups using diverse approaches (caged, split, and shielded Cas9s; protected guide RNAs) (*9*). However, the cgRNA design demonstrates the fastest kinetics thus far.

As with many cellular processes, the DNA damage response exhibits fast kinetics and involves a myriad of factors that act in a highly choreographed fashion (*10*). With vfCRISPR, Liu *et al*. found that they could achieve cleavage of half of the DNA molecules within seconds of light exposure. Mutagenesis at the cleaved sites was strictly light-dependent and efficient. With this precise control of Cas9 cleavage, the authors examined repair kinetics at various genomic sites. Modeling of repair kinetics at one target site unexpectedly suggested repetitive cleavage and repair, possibly because this site is particularly vulnerable to one type of repair product. Moreover, with time-resolved chromatin immunoprecipitation, the recruitment and retention of components of the DNA damage signaling and repair machinery could be tracked within minutes, including the rapid responder MRE11 (*11*). Spreading of a commonly used marker of DSBs, phosphorylated histone H2AX (γH2AX) (*12*), could be resolved into two rates, one estimated at ~150 kb/min and a surprising second layer at ~460 kb/min, reaching an astounding 30 Mb from the DSB within 1 hour.

Other methods to study DNA repair are less controllable. For example, ionizing radiation has long been used to follow the fast recruitment of DNA damage response factors into cytologically visible foci (*11*, *12*). However, the damage sites are not known and cannot be readily tracked over time. Moreover, ionizing radiation induces a number of different types of damage in addition to DSBs (*13*). An alternative approach has involved the hormone induction of a restriction enzyme that only makes DSBs; however, induction is not as fast as with light, and cells are not viable because of the large number of DSBs that are induced (*14*).

One elegant application of vfCRISPR involves fluorescence imaging of genomic loci (*2*). Using a Cas9–green fluorescent protein (GFP) fusion, Liu *et al*. were able to track DSBs at both alleles of a gene. They noticed that each allele had a focus of the DNA repair protein TP53-binding protein 1 (53BP1) that underwent cycles of expansion and dissolution over time, possibly due to cycles of breakage and repair. Although the duration of the initial 53BP1 cycle differed in different cells, it was well correlated between the two alleles in a single cell. Further investigation will be necessary to determine what in the cellular milieu is responsible for the correlated behavior of 53BP1.

By cytologically marking both alleles of a gene with the Cas9-GFP fusion, the authors were able to focus light onto a single allele to achieve a high degree of spatial control over DNA cleavage through the cgRNA, leading to mutagenesis of just one gene allele. Heterozygous mutations are often difficult to achieve, yet desirable when attempting to model dominant mutations or generate mouse lines for which homozygous mutations are lethal. Presumably, the focused application of light will also prevent off-target activity.

Light-directed Cas9 activation is expected to be transformative for understanding the kinetics of the cellular response to DSBs. The exquisite spatial control of DSB formation by light could also be exploited to illuminate repair pathways in different nuclear compartments. It also provides flexibility in experimental design to introduce DSBs at multiple locations individually, sequentially, or simultaneously. Within organisms, adapted optogenetics (light-activated protein manipulation) in neurons could allow precise interrogation not only of gene function, but also of the role of DNA breaks in neuronal activity and repair pathways. Moreover, Cas9 and other CRISPR proteins have been repurposed beyond DSB induction, including for the introduction of other types of DNA lesions. How well light activation through cgRNAs translates to studying other processes remains to be seen, but there is promise for precise interrogation of other DNA repair pathways. ■

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ELECTROCHEMISTRY

Electrification of the chemical industry

Performing chemical synthesis with renewable electricity can reduce carbon emissions

By **John L. Barton**

Curbing carbon emissions while maintaining quality of life is a global challenge for manufacturing processes that will require process innovation. One approach is replacing energy from the burning of carbon-based fuels with energy supplied by "green" electrons. This goal can be achieved in some cases by simply replacing heat supplied by combustion with electrical heating (*1*). In chemical synthesis, it can also more elegantly supply reaction energy through electrochemistry. On page 1228 of this issue, Leow *et al*. (*2*) propose an electrochemical route to ethylene oxide (EO) and propylene oxide (PO) that promises cleaner, more efficient, and more selective processing. Ethylene and propylene were epoxidized electrochemically to EO and PO, respectively, at industrially relevant current densities with Faradaic (electron-specific) selectivities ~70% to the target epoxide (*2*).

Leow *et al*. coupled an electrochemical flow cell to homogeneous reactions for an overall reaction, $C_2H_2 + H_2O \rightarrow C_2H_2O + H_2$, for EO synthesis (see the figure). Two electrochemical reactions drive this reaction. Chlorine evolution occurs at the anode, $2Cl^- \rightarrow 2e^- + Cl_2$, and hydrogen evolution occurs at the cathode, $2H_2O + 2e^- \rightarrow H_2 + 2OH^-$, where e is the charge on the electron. These reactions are not particularly interesting; what is innovative is coupling these two simple reactions with three subsequent, homogeneous chemical reactions. Dissolved chlorine in the anodic solution dissociates into hydrochloric and hypochlorous acid (HCl and HOCl, respectively). The latter reacts with ethylene to make 1-chloro-2-hydroxyethane ($HOCH_2CH_2Cl$), or ethyl-

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