

# Shining Light on CRISPR Gene Editing



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## Advanced spatiotemporal control of CRISPR-Cas9 activity is demonstrated through the use of chemically modified photoactivatable guide RNA.

In order to understand a biological system, it is a great advantage if you can perturb it in a controlled manner. The Stevens group has achieved this by spatiotemporal control of gene regulation using a simple photoactivated CRISPR guide RNA (gRNA) construct bound to Cas9 protein.<sup>1</sup> Historically, it has been challenging to modify the function of genes, that is, until the landmark discovery of the CRISPR–Cas9 system.<sup>2</sup> The Cas9 protein, a DNA cutting endonuclease, generates blunt double-strand DNA breaks in the genome that are recognized by the cell and repaired imperfectly, causing gene editing. What is remarkable about the system is that Cas9 is directed to its DNA target by a gRNA molecule through Watson–Crick base pairing. Crucially, these gRNAs are relatively straightforward to produce in high yield and purity by enzymatic transcription or solid-phase synthesis methods making almost any DNA target accessible.

At present, many Cas9 protein modifications are known that reduce DNA cutting at unwanted sites,<sup>3</sup> allow the transport of other proteins (e.g., gene activators or fluorescent proteins) to DNA targets, and even enable single point mutation of the genome.<sup>4</sup> For fundamental studies, it would be useful to convert these systems into inducible ones.

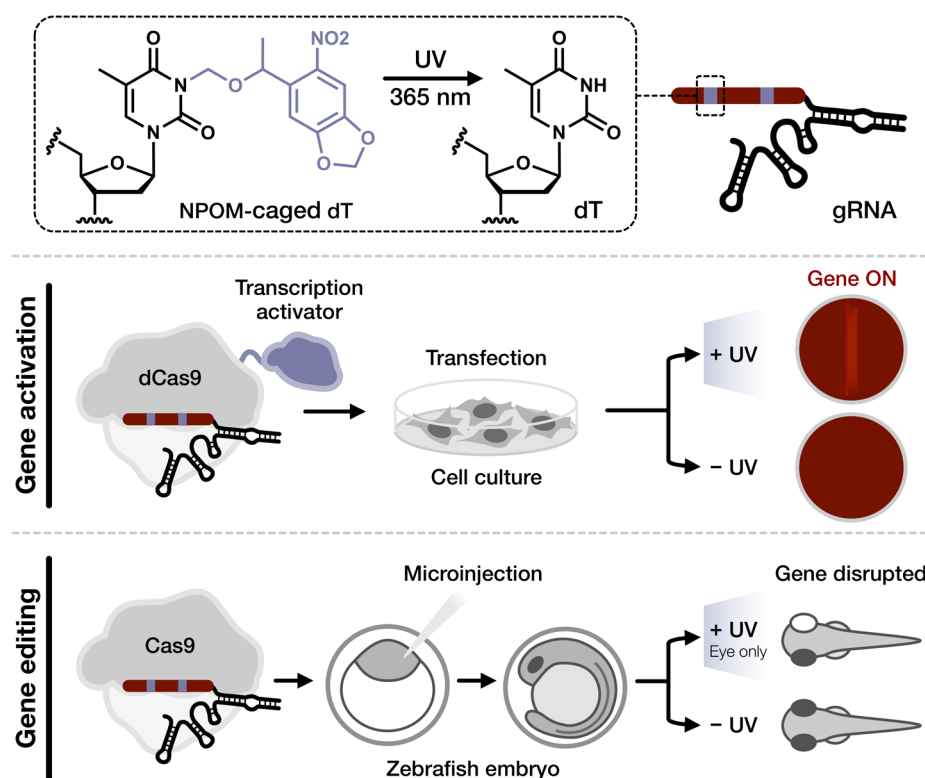
When the Cas9–gRNA complex is introduced into an organism, its time of arrival at the site of action will vary from cell to cell. Hence, gene editing will occur in different cells at varying times, and a stochastic effect is observed. However, to unravel a complex network of gene interactions, precise control is required at specific locations and at exact times during the development of the organism.

Although a variety of photoactivatable CRISPR systems have been developed to achieve this, they require extensive protein engineering<sup>5</sup> and need a strong light stimulus that reduces cell viability.<sup>6</sup> Crucially, these systems are not all compatible with the various Cas9 protein modifications described above. These limitations have now been addressed. The Stevens group, and independently the Deiters group,<sup>7</sup> have described a simple chemical modification of the gRNA that allows spatiotemporal control of CRISPR–Cas9 gene editing. The methodology involves the use of photocleavable blocking groups attached to selected nucleobases in the protospacer DNA targeting region of gRNA. These blockers prevent the gRNA from binding to its DNA target by inhibition of Watson–Crick base pairing. The CRISPR–Cas system can be delivered into cells or organisms by techniques such as lipofection after which it will find its way into the nucleus. Irradiation of the cells at a wavelength that does not damage DNA or RNA leads to cleavage of the blocking groups. Binding of the protospacer region of the unblocked gRNA to its DNA target occurs, and Cas9 nuclease activity is restored. Hence, gene editing can be “switched on” by light at any time up to several days after the initial transfection.

Although the chemistry used in this approach is not new and photocleavable nucleobase blocking groups have been around for several years,<sup>8</sup> this is the first time they have been applied to CRISPR. Interestingly, the Stevens group placed the photocleavable 6-nitropiperonyloxymethyl (NPOM) blocking group on a DNA T monomer in a split (bimolecular) crRNA–tracrRNA format (36 and 67 bases long RNAs), whereas the Deiters group put the blocking group on RNA

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**Figure 1.** Stevens group prepared guide RNAs with photocleavable caging groups, which upon nontoxic UV irradiation are removed to allow activation of the CRISPR–Cas9 system. This enables gene activation and disruption to be spatiotemporally controlled in both cell culture and live zebrafish embryos.

G and U monomers in a single (unimolecular) 100-mer gRNA. The Stevens group's work builds on studies that show DNA nucleotides are tolerated in the gRNA, allowing the use of synthetically more accessible DNA monomers. Their choice of a split system was dictated by the practical considerations of higher yields and lower costs of shorter RNA molecules made by solid-phase synthesis. Such RNA constructs are available commercially. However, the unimolecular gRNA system, although more challenging to synthesize, provides a more efficient gene-editing construct. To their credit, the Deiters group carefully analyzed their modified 100-mer synthetic RNA constructs to demonstrate their purity. This provides the reader with confidence in the subsequent biological studies.

Stevens demonstrated the power of RNA-level photoactivation by performing: (1) DNA target cleavage *in vitro*, (2) gene editing in cells, (3) selective cellular activation of fluorescent proteins, and (4) selective gene editing in zebrafish embryos (Figure 1). The last example is fascinating; photoactivation at a particular stage of the development cycle and at a particular location resulted in one eye of the embryo being selectively mutated. Ultimately, the time window in which photoactivation can achieve high levels of gRNA activity is limited by the stability of the gRNA and its steady dilution upon cell division. For one-off events such as gene editing,

the time of photoactivation is less important than for gene activation in which continuous binding of gRNA to its DNA target is desirable. The photocleavable blockers do not stop unintended DNA cutting at incorrect sites, but they do not make it worse. These unblocked gRNAs remain active post-activation and post-gene editing, a fundamental cause of off-target activity.

The results from the Stevens and Deiters groups are convincing, and the fact that these two independent groups have arrived at similar conclusions is particularly reassuring. In the future, deeper biochemical and genome-wide studies are desirable. As a point of detail, the Stevens group used only two photocleavable blockers in their gRNAs, whereas Deiters chose to employ four blocked nucleotides. Two modifications are sufficient to produce the desired nuclease inhibition, and leaky activation in the absence of irradiation is minimal, but it is not negligible. More systematic and

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deeper studies of the optimal number and position of modifications will be useful to facilitate the more general use of the gRNA.

Overall, these caged gRNAs are highly promising tools for spatiotemporal control of gene editing. They should enable the investigation of complex physiological events and provide a better understanding of dynamic gene regulation. In principle, multicolor targeting systems could be developed to allow different genes to be switched off simultaneously or at different times. Moreover, this strategy could be applied to other CRISPR–Cas that have distinct properties (e.g., Cas13).<sup>9</sup> It remains to be seen how members of the vast biological community working on gene editing will take advantage of this exciting technology. Finally, the photo-activated systems described by Stevens and Deiters have to be made by chemical synthesis; the crucial site-specific RNA modifications cannot be introduced by transcription. This is another example of the power of the solid-phase phosphoramidite method made possible by the pioneering work of Caruthers and others.<sup>10</sup> At the end of the day, it is all chemistry.

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