A new world order: Tailored gene targeting and regulation using CRISPR (Editorial)

A New World Order: Tailored Gene Targeting and Regulation Using CRISPR

Marc S Weinberg and Kevin V Morris

Not since the discovery of RNA interference has a new technology made such a powerful impact in the molecular biosciences. The RNA-guided, modified type II prokaryotic clustered regularly interspaced palindromic repeats system (CRISPR) represents not only an exceptional tool but also potentially an important new therapeutic modality.

CRISPR, in its native function, provides adaptive immunity in bacteria by introducing targeted DNA mutations in pathogenic viruses and plasmids (re-viewed in ref. 1). The breakthrough technology came in 2012 with the development of modified CRISPR components that worked in human cells. This simplified system was found to be easily adapted into expression vectors to target any DNA sequence from virtually any organism, thereby greatly expanding its function and utility. To date this system has been used in human cells to target gene excision, activating and repressing proteins, as well as to instill gene fusions.

CRISPR has the advantage over other gene excision approaches, such as those based on transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs), in that a small guide-RNA component is easily programmable and remains physically separate from Cas9/dCas9 expression.

CRISPR/Cas9 might prove to be a useful future therapeutic option for the targeting, editing, or inactivation of disease gene sequences, whether inherited or acquired. Two impressive studies point to powerful therapeutic possibilities for this technology. Schwank and colleagues applied CRISPR/Cas9 to target and correct the CFTR locus in cultured intestinal stem cells of cystic fibrosis patients.2 The corrected allele was shown to be fully functional in clonally expanded intestinal organoids. In a second study, a dominant inherited dis-ease was corrected in zygotes of mice carrying mutations in a cataract-causing gene, Crygc.3

Few question the value of CRISPR/Cas9 as a genomic editing tool. However, its therapeutic potential is less certain—at least, at this time. Two overriding concerns are immediately apparent. First, it is unclear whether expression of this nonhuman protein will be tolerated. For example, expression of exogenous marker proteins such as green fluorescent protein can elicit substantial unwanted immunological responses.4 Second, despite efforts aimed at reducing off-target effects, previous studies with endonucleases have shown that even minor nonspecific cleavage of DNA can result in chromosomal deletions and rearrangements as well as cellular damage. Moreover, there is the possibility of targeting closely related gene sequences. Although efforts should be made to address

these concerns, contingencies are already available that can address safety-related issues for therapeutic applications. For example, a “hit and run” approach may prove beneficial where exposure to CRISPR/Cas9 is highly restricted or where cells can be extensively screened ex vivo before their therapeutic use. An example of this option is the targeting of HIV-1 infection. Proof-of-concept studies have already used CRISPR/Cas9 to induce targeted deletions of the HIV-1 co-receptor CCR5 (refs. 5, 6) as well as the integrated HIV-1 promoter (ref. 7).

The modified CRISPR/Cas9 system is undoubtedly a powerful and far-reaching tool for biology and medicine. A wide range of functions has been identified, and one can envision many more potential functions in which guide RNAs recruit factors to a specific genomic address. Clearly, the therapeutic application of CRISPR/Cas9 is still in its infancy, but with the astonishing pace of discovery one can only wonder what the next 18 months have to offer.

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