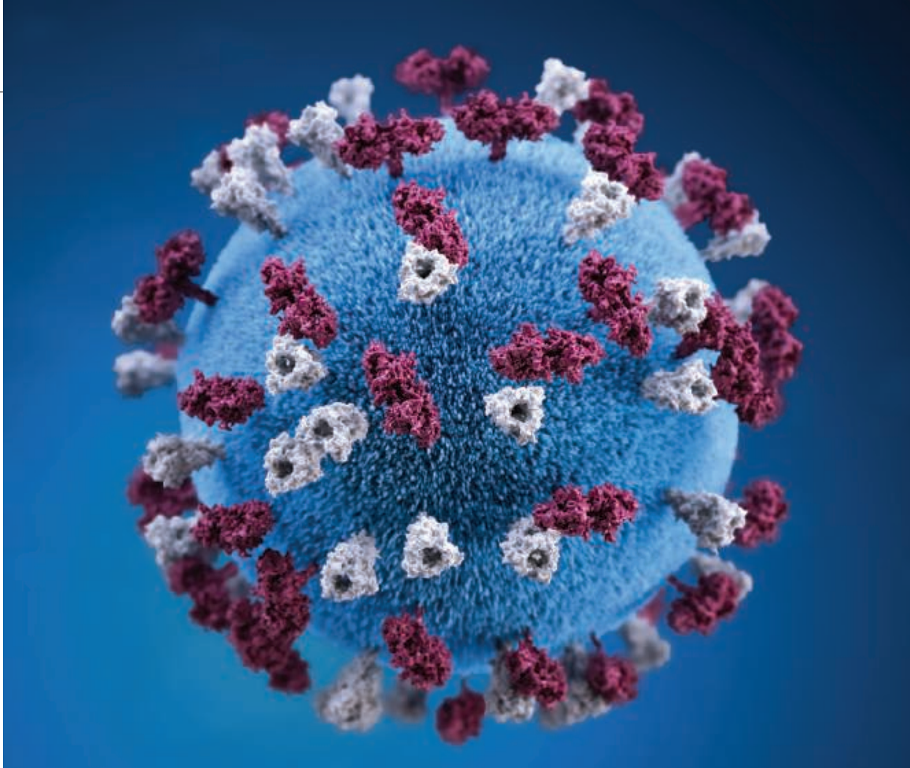

Understanding how measles virus and related viruses fuse with host cells could reveal therapeutic opportunities.

conserved receptor-binding steps that G, H, or HN undergo to trigger F (*8*–*10*). Furthermore, it was recently shown for NiV that interactions between F and G can modulate membrane fusion steps beyond F triggering. For example, mutations in G that strengthen F–G interactions can allow F triggering and even fusion pore formation but hinder fusion pore expansion in a cell–cell fusion assay, and fusion kinetics data also point to F–G interactions playing roles beyond F triggering (*11*). The structure of a new intermediary step in the F conformational cascade that leads to membrane fusion identified by Zyla *et al.* is an important finding, because every step discovered in this process becomes a potential druggable target.

Most prior cryo-EM structures of type 1 fusion proteins are primarily of prefusion-stabilized F proteins, with cysteines and/or proline substitutions that prevent pre- to postfusion transitions (*12*–*15*). The designed F_{ECTO} construct retains the ability to transition beyond the prefusion state, and the intermediate form observed is consistent with prior descriptions of postfusion conformations. As such, F_{ECTO} constructs provide an ideal platform to further mechanistically characterize the paramyxoviral fusion cascade with high-resolution structural data, which will greatly improve understanding of the infection process and reveal potential therapeutic targets.

Zyla *et al.* reveal a new structural conformation for MeV F, a new platform to study such structures for other paramyxoviruses (and possibly type 1 fusion proteins of other viruses, including influenza viruses or coronaviruses), a new potential mAb therapeutic for MeV, and a new potential druggable target for other paramyxoviruses. It remains to be seen whether similar findings will apply to other paramyxoviruses. However, given the conservation of the mutated region, it is highly likely that these findings will have broad repercussions. It is also unclear whether these structures will be similar in the presence of the respective H for MeV, and HN, or G for other paramyxoviruses. An additional hurdle will be to ratify these structures in the context of actual viral particles. The disease burden caused by paramyxoviruses, including human MeV, MuV, HPIV-3, and NiV, and animal viruses is high. This study increases the potential discovery of mAbs to treat and prevent infection by these and potentially related viruses. ■

ILLUSTRATION: ALISSA ECKERT/CDC/PUBLIC DOMAIN

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MEDICINE

An epigenetic editor to silence genes

A new class of editor has improved delivery, durability, tunability, and safety

By **Madelynn N. Whittaker** *and* **Kiran Musunuru**

Although the practice of molecular medicine has taken enormous strides in recent years—as attested to by a wave of approvals of antisense oligonucleotide (ASO) therapies as well as the first CRISPR-based therapy—neurodegenerative diseases, such as prion disease, Huntington's disease, Alzheimer's disease, and Parkinson's disease, remain a difficult challenge. Toxic protein aggregation has been implicated in neurodegeneration, pointing to gene silencing as a broadly applicable therapeutic strategy. Although ASOs and CRISPR-based silencing offer potential to suppress the expression of pathogenic proteins, efforts have not yet been successful. On page 1421 of this issue, Neumann *et al.* (*1*) report a new epigenetic editor that can silence the expression of prion protein (PrP) in the brains of mice and offers a fresh approach to the treatment of neurodegenerative diseases.

ASOs impair protein synthesis by binding a target mRNA and inducing its degradation, splicing modifications, and other modifications that alter its availability for translation. Although preclinical efficacy has been demonstrated with ASO therapies—e.g., for prion disease in a mouse model (*2*)—success in the clinic has been either lacking or modest so far. ASOs also have substantial technical limitations, including their short-lived efficacy requiring repeated intrathecal injections (into the spinal canal) and their risk of adverse drug reactions, such as hydrocephalus, meningitis, and hepatotoxicity (*3*). CRISPR-based silencing techniques offer an alternative approach, tethering a catalytically dead Cas protein to effector domains. Initial attempts at "epigenetic editing" used repressor domains that proved capable of only transient gene silencing (*4*). CRISPRoff, a recently developed epigenetic editor that

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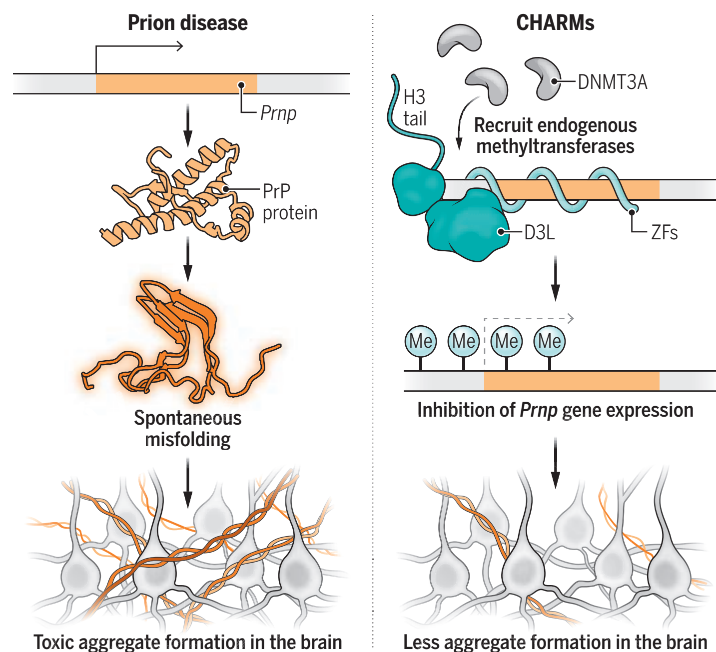
contains a DNA methyltransferase domain, can achieve more-durable gene silencing through site-directed methylation of promoters (*5*). Despite this advancement, important limitations remain: Adeno-associated virus (AAV) vectors remain the primary means of delivery of transgenes to the brain; CRISPRoff is too large to fit in a single AAV vector; AAV vectors promote long-term editor expression, incurring the risks of deleterious immune responses and off-target editing; and the tethering of constitutively active effector enzymes can lead to unrestrained off-target effects and cytotoxicity.

Neumann *et al.* present coupled histone tail for autoinhibition release of methyltransferase (CHARM), a compact, enzyme-free epigenetic editor that can recruit an endogenous DNA methyltransferase, induce hypermethylation of a target gene promoter, and durably silence expression of the gene and thus production of its protein product in the brains of mice upon delivery via a single AAV vector (see the figure). This innovative approach addresses each of the limitations of existing editing approaches.

Epigenetic editing in neurodegeneration

The CHARM system silences the *Prnp* promoter, reducing PrP expression levels in the brains of mice. This system involves targeting specific DNA sequences with ZFs coupled to D3L and an H3 tail, which recruit and activate endogenous DNMT3A. This results in targeted DNA hypermethylation and thus gene silencing, and the system can be delivered in a single AAV vector.



AAV, adeno-associated virus; CHARM, coupled histone tail for autoinhibition release of methyltransferase; DNMT3A, DNA methyltransferase 3A; D3L, DNMT3L C-terminal domain; H3, histone H3; Me, methyl group; *Prnp*, prion protein gene; PrP, prion protein; ZFs, zinc fingers.

The delivery of CRISPR-based editing tools in vivo is challenging because of their complexity and size. Although mRNA-containing lipid nanoparticles can efficiently deliver large editors into the liver (*6*, *7*), they are currently limited in their ability to target other organs, such as the brain. To achieve efficient nonliver delivery, multiple AAV vectors are required to accommodate the large size of epigenome editors, such as CRISPRoff (*8*). But this strategy means lower potency, increased AAV doses to compensate, and thus increased toxicity. By pivoting away from the use of CRISPR and creating a modular zinc finger–based "ZFPoff" epigenome editor that does not require large protein domains—zinc finger DNA binding motifs are much smaller than Cas proteins—Neumann *et al.* fit the editor into a single AAV vector.

The use of tethered enzymes can increase editing potency but can have undesirable consequences. A well-established example is guide RNA–independent off-target editing by CRISPR base editors, where the tethered deaminase domain can stochastically alter both DNA and RNA sequences throughout the genome and transcriptome (*9*–*11*). Neumann *et al.* confirm that cytotoxicity is an issue with the constitutively active methyltransferase domain [D3A, derived from the mammalian DNA methyltransferase 3A (DNMT3A) protein] used in CRISPRoff, especially when used with zinc fingers in ZFPoff. Taking their cue from the natural mechanism of DNMT3A regulation, Neumann *et al.* remove the D3A domain from their editor and use a fusion of the nonenzymatic DNMT3L C-terminal domain (D3L) and a histone H3 tail, which together recruit endogenous and inactive DNMT3A specifically to the target genomic site and activate it in situ, avoiding any generalized cytotoxicity. The D3L–H3 tail fusion is quite small, which means that up to three orthogonal epigenetic editors (targeting different genomic regions) can fit into one AAV vector with some clever engineering involving split inteins.

Neumann *et al.* overcome concerns about potential adverse effects of long-term expression of the editing tool by demonstrating the ability to self-silence expression of the epigenetic editor by having one of the editors target the promoter in the AAV vector, thereby turning off the expression of all editors. CHARMs can be programmed to tune this self-silencing to optimize the balance between therapeutic gene silencing and the transience of editor expression. Yet another safety advantage is that CHARMs lack bacterial epitopes (unlike CRISPR-based tools), so their immunogenicity profile could be more favorable compared with those of other epigenetic editors.

Neumann *et al.* establish the efficacy and durability of CHARM-mediated epigenetic editing when applied to the PrP protein in vivo. AAV delivery of CHARMs—including self-silencing CHARMs—targeted to the *Prnp* promoter achieved widespread reductions in *Prnp* transcripts and PrP protein levels throughout the brain, with hypermethylation in the *Prnp* promoter for up to 13 weeks after treatment. Based on previous work (*2*), such effects are predicted to extend survival in mouse models of prion disease and perhaps in human patients as well.

With the development of CHARMs, Neumann *et al.* have introduced a potent and safe editing technology for gene silencing via AAV delivery into otherwise difficult-to-target organs, such as the brain. Limitations include uncertainty about the long-term durability of gene silencing through methylation for *PRNP* and other disease loci in humans, which is not assured and remains to be established on a case-by-case basis (*12*). Additional challenges to overcome are that gene silencing is likely to be a useful therapeutic strategy only for a subset of diseases, and the potential for off-target editing incurred with zinc fingers is less well-defined than that with CRISPR-based tools. Nonetheless, epigenetic editors akin to CHARMs may ultimately prove to be as impactful for human health as other transformational technologies, such as base editing and prime editing. ■

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