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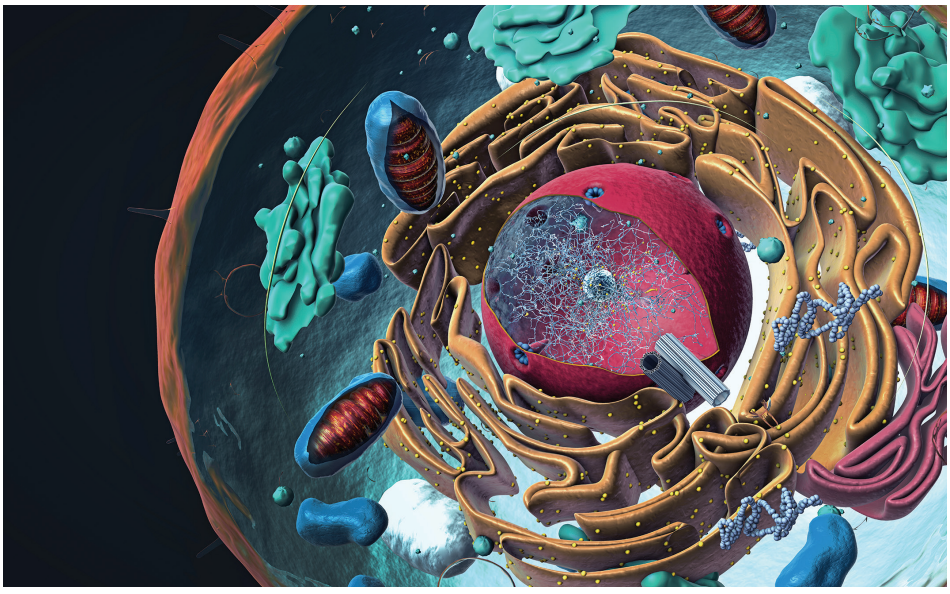
HIJACKING CELLULAR MECHANISMS TO TREAT COMPLEX DISEASES

INTRODUCTION

Genetic disorders are frequently associated with overactive, underachieving, or otherwise defective protein function. Disease treatments have often focused on small-molecule drugs that target a protein's active site. But many proteins fulfill a structural role and hence lack active sites or enzymatic activity. As a result, many protein targets associated with genetic disorders are effectively undruggable using active site–based therapies.

As an alternative to modulating the activity of enzymatic proteins, researchers are exploring ways to modify cellular protein levels. One powerful approach



Scientists are developing novel treatments using the cell's own regulatory machinery to go after previously undruggable targets.

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is to employ the cell's own regulatory machinery to go after difficult targets. For example, using short complementary RNA, scientists can repress and in some cases amplify the expression of proteins by regulating messenger RNA (mRNA). Alternatively, targeted protein degradation has shown great promise in repurposing cellular machinery to remove proteins. Both of these mechanisms offer an alternative to traditional protein antagonist or agonist drugs and have the potential to provide new medical breakthroughs for previously untreated or undertreated diseases.

That just a handful of drugs in either category have been approved for use highlights some level of risk for small pharmaceutical companies. However, biotech partners with expertise in these modalities and overarching biology can be the key to achieving success with these mechanisms.

THE CURRENT DRUG DISCOVERY PARADIGM

The types of molecules available to drug researchers are limited by existing chemistry techniques and knowledge of biological mechanism. As such, in modern medicine, therapeutic molecules have tended to focus on the modulation of protein activity. Statins—top-selling medications that lower cholesterol by inhibiting a particular enzyme and blocking lipid biosynthesis—are a prime example of traditional small-molecule drugs.[1]

Traditional small-molecule drugs work by interfacing with proteins at their active site, a region within the protein often likened to a pocket. Active sites are optimized for interacting with specific substrate or target molecules. Drug classes targeting enzymatic pockets can block normal functions and thus alter protein activity. These protein antagonist and agonist mechanisms were historically a major focus of pharmaceutical research. Craig Crews, the John C. Malone Professor of Molecular, Cellular and Developmental Biology at Yale University, describes this as an occupancy-driven paradigm of pharmacology. "Normally, you have an active site and you have an inhibitor, and you need to fill that active site," Crews says. "You might need to fill 98–99% of those active sites to get the clinical benefit."

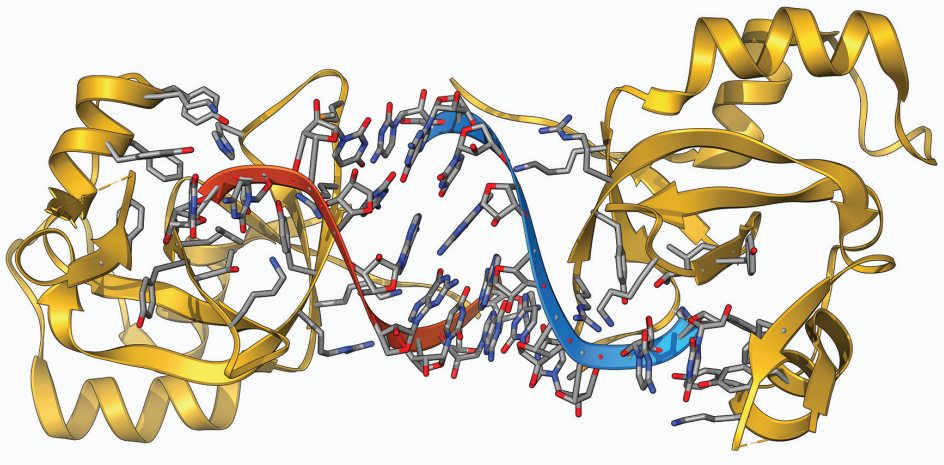
One popular approach is to go after "easier" classes of targets, including kinases and G-protein-coupled receptors (GPCRs).[2] "There is a perception that since these kinds of targets are easier to drug, new targets outside of this class are too difficult to pursue," says Sajesh Parathath, senior director of biology at WuXi AppTec. This perception is supported by the many approved therapies that target kinases and GPCRs, as well as by the fact that developing novel drugs directed at these enzymes remains an "active and fruitful area of research," he adds. But Parathath points out that pharmacological approaches based on targeting a protein's active site can be limiting, as many proteins "don't really have these classic druggable pockets." How to target such proteins is an emerging and compelling challenge in pharmacology. To address it, scientists are developing multiple strategies that target existing biological pathways to increase or lower the level of protein within the cell, offering new treatment possibilities for previously undruggable targets.

THERAPEUTIC RNA: NIPPING PROBLEMATIC PROTEINS IN THE BUD

It would be impossible to discuss RNA-based therapies in 2021 without mentioning the US Food and Drug Administration's emergency use authorization and subsequent widespread use of two mRNA-based vaccines against COVID-19. Though RNA therapies previously struggled to gain credibility, the vaccines' efficacy has scored favorability points. After years of skepticism on the part of the pharmaceutical industry as to whether RNA-based therapies were worth pursuing, the field appears to have reached a tipping point.[3]

In some ways, mRNA translation—a protein's origin—is the most obvious place to explore when attempting to therapeutically alter cellular protein levels. However, scientists now realize that the central dogma of protein expression is more complicated than mRNA's straightforward ferrying of genetic information to the ribosome. The discovery in 1998 that noncoding RNA molecules could silence the expression of proteins after transcription was a revelation and led to the invention of RNA interference (RNAi) therapies. Short, noncoding nucleic acid sequences, complementary to a portion of an untranslated region of a target mRNA, can bind to the mRNA and prevent it from being translated into a protein. This method of posttranscriptional control occurs in human cells in the form of microRNAs (miRNAs). In the 2 decades since this mechanism was discovered, researchers have designed small interfering RNA (siRNA) therapies, which mimic the function of miRNAs in order to target a troublesome gene for suppression.[4,5]

Patisiran, the first RNAi drug, was approved in 2018 to treat the hereditary neurodegenerative disease hereditary transthyretin-mediated (hATTR) amyloidosis, in which a mutation in the gene for the protein transthyretin (TTR) causes the protein to misfold and aggregate in tissues. Patisiran binds to the



RNA-induced silencing complex (RISC), molecular model. This complex consists of a bacterial argonaute protein bound to a small interfering RNA (siRNA) molecule (red and blue). RISC is the multiprotein complex responsible for the gene silencing process known as RNA interference (RNAi). This involves one strand of siRNA, used as a template for recognising complementary mRNA (messenger RNA), and an argonaute protein that catalyses the RNA interference process. This complex plays a role in gene regulation and defence against viral infection.

Image credit: Laguna Design / Science Photo Library

mRNA for TTR, marking it for degradation and thus reducing the amount of the protein that is expressed.[4] A second RNAi drug, givosiran, gained approval in 2019 to treat acute hepatic porphyria (AHP). The condition manifests in the liver, where the important biomolecule heme is synthesized, and is characterized by a harmful accumulation of heme synthesis intermediates. Givosiran reduces the expression of a liver enzyme that catalyzes an early step in the heme synthesis pathway, with the result that fewer of the intermediate molecules that cause AHP symptoms are produced.[6]

Both drugs are classified as siRNAs, about 21 or 22 nucleotides long, which are complementary to a region in the mRNA for a protein targeted for suppression. In combination with the cell's own transcription-regulating machinery, siRNAs form an RNA-induced silencing complex (RISC). RISC uses the complementarity of the siRNA sequence to bind to the mRNA, preventing translation and in some cases causing degradation.[4,7] There are other examples of RNA-based regulation that are both nuanced and orthogonal.

A single miRNA can potentially suppress several proteins, resulting in the modification of biological pathways versus single targets. For example, miR-33 can regulate several proteins involved in cholesterol metabolism.[8] From a drug development perspective, overexpression of an miRNA could amplify its natural function while anti-miRNA, strands of RNA that bind to miRNA, could be used to negate it.

Small activating RNAs (saRNAs) are similar to siRNAs in terms of nucleotide length and other features. These targeted RNA molecules bind argonaute-2 and identify complementary promoter regions of select genes and bring about the overexpression of proteins through several mechanisms.[9] Although not all proteins can be upregulated using saRNA, the discovery of this mechanism has opened up another level of regulation that can be exploited. MiNA Therapeutics is developing an saRNA called MTL-CEBPA to treat liver cancer, among other indications, by directly activating the transcription of CCAAT (cytosine-cytosine-adenosine-adenosine-thymidine)/enhancer-binding protein alpha (CEBPα), a protein that regulates myeloid cells.

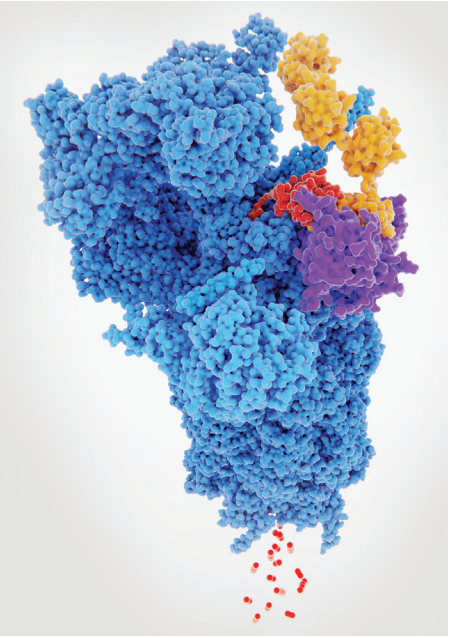
TARGETED PROTEIN DEGRADATION: CLEARING PROTEINS FROM THE CELL

Another approach focuses on the removal of disease-causing proteins from the cell. Just as with RNA therapeutics, the pharmaceutical industry was initially uncertain about the utility of targeted protein degradation, but in recent years the science has begun to attract more attention.[10]

After proteins have carried out their function, they are rendered back to their constituent amino acids by a suite of cellular machineries evolved for that purpose. This process allows the cell to recycle amino acids into new proteins. It also serves as a quality control method for proteins and is a way to quickly reduce protein levels.[11] These actions are executed by the ubiquitin-proteasome

system (UPS). The keystone components of the UPS are the E3 ubiquitin ligases, a family of enzymes responsible for recognizing proteins destined for degradation and tagging them with ubiquitin.[11,12]

In an interesting case, the blood cancer therapeutic lenalidomide, which the FDA approved despite an incomplete understanding of its mechanism of action, was retrospectively found to work through hijacking the UPS. The drug binds to an E3 ligase, triggering the ubiquitin tagging and degradation of transcription factors that regulate hematopoietic cell differentiation.[13] In an effort to repurpose the UPS system for therapeutic ends in a more deliberate fashion, researchers invented the proteolysis-targeting chimeras (PROTACs).[14]



The ubiquitin-proteasome system. Image shows a proteasome degrading a protein (red) tagged with polyubiquitin (yellow). Source PDB entry 5GJQ. 3d rendering.

Image credit: Shutterstock

PROTACs consist of two small ligands held together by a linker, one end targeting the protein destined for degradation and the other targeting an E3 ligase. The PROTAC coaxes both proteins into close proximity, where the target protein is tagged with ubiquitin. The advantage of this transient association is that the ligands need not be optimized to fit snugly into the active site of either protein, which dramatically increases the number of proteins that can be targeted for degradation.[14]

The PROTAC drug candidate ARV-110 is in clinical trials for prostate cancer. The proposed mechanism for ARV-110 is recruitment of an E3 ligase to the androgen receptor protein, thereby reducing overall receptor expression in cells. Targeting overall androgen protein levels rather than binding to a specific active site on the target protein means the risk of developing resistance—a common issue with prostate cancer drugs—could be avoided. Furthermore, since the engagement of the PROTAC molecule with ligase and target protein may be transient and reversible, Crews and colleagues refer to this catalytic activity of PROTACs as an event-driven paradigm of pharmacology as opposed to the standard occupancy-driven paradigm.[10,12]

Scientists are taking advantage of other pathways that can lead to protein degradation and overcome some of the limitations of PROTAC molecules, such as not being able to target extracellular or transmembrane proteins. Some biological pathways lead to the lysosome, a membrane-bound organelle that degrades proteins, and scientists are beginning to identify molecules that can target

disease-causing proteins to such pathways.[15] For example, researchers recently identified compounds that sent mutant huntingtin protein to the lysosome.[16] Countless pathways, regulatory nodes, and fundamental biology are waiting to be discovered and applied to the treatment of diseases. Finding the balance between using the cell's biology and disrupting it to a detrimental level will be key.

EXPLORING NEW DRUG MECHANISMS AND MODALITIES

Craig Lindsley, professor of pharmacology at Vanderbilt University, notes that probing novel mechanisms and drug discovery paradigms requires time and resources. "The biggest issue is the unknown, and it may take a significant investment in basic science to understand the pharmacology," he says. "For companies small and large, the time commitment may be prohibitive."

Until these new modalities and mechanisms have been proved successful, they offer the pharmaceutical industry no guarantees. "Once a drug modality has been approved, it opens the door to being a validated mechanism of action," Parathath says. But someone has to take the first leap into that area. This might be especially difficult for companies that in the past focused entirely on small molecules. New drug discovery paradigms bring new challenges in scaled-up manufacturing, formulation, stability, and safety.[17]

WuXi AppTec is making an effort to lower some of these barriers, according to Parathath. Many companies lack the capability and scalability needed to launch drug discovery programs in new areas of biology, he says, and "WuXi AppTec is trying to make it possible for a company of any size to use our services to enable them to test any possible mechanism."

"Very few companies actually work with RNA-based therapies," Parathath says. "In fact, the majority of the siRNA research is done by four major companies," and there's room for more in that mix, he adds. "WuXi AppTec's vision is that every drug can be made and every disease can be treated." The company aims to leverage its extensive experience and integrated global networks to help diverse organizations utilize novel mechanisms and modalities in their research.

CONCLUSION

Novel drug mechanisms have continued to bear fruit in the form of a much greater diversity of approved compounds.[3] In the field of targeted protein degradation, two PROTAC drug candidates entered clinical trials in 2019, and research in the field continues unabated.[14] A small selection of RNA-based therapies have been approved; many more are in the pipeline, taking aim at a number of cancers as well as liver and nervous system diseases. The success of RNA-based vaccines in terms of therapeutic outcome and speed have made the idea of RNA-based therapies a more palatable approach for scientists, regulatory agencies, and the general public.

The immense complexity of human biology means that even the hundreds of approved therapies target only a very small percentage of our proteome. "It's

really amazing how much information is actually in a cell," Parathath says. "The level of sophistication for regulation and control of all of the functions is unbelievable when considering all the interactions are integrated and layered on top of each other at a microscopic level." As the understanding of cellular biology continues to increase, scientists are able to harness newly discovered mechanisms and systems to treat diseases. This can lead to a more purposeful approach to drug design: with more therapeutic modalities and mechanisms in their arsenal, scientists can select an approach because of its unique strengths instead of previous success. The stage is set for an explosion of new ideas, expanding technologies, novel modalities, and the willingness to invest in novel mechanisms to take on and be successful in treating complex diseases.

REFERENCES

1. Camelia Stancu and Anca Sima, "Statins: Mechanism of Action and Effects," *J. Cell. Mol. Med.* 5 no. 4 (Oct. 2001): 378-87, https://doi.org/10.1111/j.1582-4934.2001.tb00172.x.
2. Peng Wu, Thomas E. Nielsen, and Mads H. Clausen, "FDA-Approved Small-Molecule Kinase Inhibitors," *Trends Pharmacol. Sci.* 36, no. 7 (July 1, 2015): 422–39, https://doi.org/10.1016/j.tips.2015.04.005.
3. Feng Wang, Travis Zuroske, and Jonathan K. Watts, "RNA Therapeutics on the Rise," *Nat. Rev. Drug Discov.* 19, no 7 (April 27, 2020): 441–42, https://doi.org/10.1038/d41573-020-00078-0.
4. Sarah Bajan and Gyorgy Hutvagner, "RNA-Based Therapeutics: From Antisense Oligonucleotides to miRNAs," *Cells* 9, no. 137 (Jan. 7, 2020): 137, https://doi.org/10.3390/cells9010137.
5. Ryan L. Setten, John J. Rossi, and Si-ping Han, "The Current State and Future Directions of RNAi-Based Therapeutics," *Nat. Rev. Drug Discov.* 18 (June 2019): 421–46, https://doi.org/10.1038/s41573-019-0017-4.
6. "Second RNAi Drug Approved," news brief, *Nat. Biotechnol.* 38 (April 7, 2020): 385, https://doi.org/10.1038/s41587-020-0494-3.
7. Jenny K.W. Lam et al., "siRNA versus miRNA as Therapeutics for Gene Silencing," *Mol. Ther. Nucleic Acids* 4, no. e252 (Jan. 1, 2015), https://doi.org/10.1038/mtna.2015.23.
8. Katey J. Rayner et al., "MiR-33 Contributes to the Regulation of Cholesterol Homeostasis," *Science* 328, no. 5985 (June 18, 2010): 1570–73, https://pubmed.ncbi.nlm.nih.gov/20466885/.
9. Debashis Sarker et al., "MTL-CEBPA, a Small Activating RNA Therapeutic Upregulating C/EBP-α, in Patients with Advanced Liver Cancer: A First-in-Human, Multicenter, Open-Label, Phase 1 Trial," *Clin. Cancer Res.* 26, no. 15 (Aug. 1, 2020): 3936–46, https://doi.org/10.1158/1078-0432.CCR-20-0414.
10. Asher Mullard, "Targeted Protein Degraders Crowd into the Clinic," *Nat. Rev. Drug Discov.* 20, no. 4 (April 2021): 247–50, https://doi.org/10.1038/d41573-021-00052-4.
11. Matthieu Schapira et al., "Targeted Protein Degradation: Expanding the Toolbox," *Nat. Rev. Drug Discov.* 18, no. 12 (Dec. 2019): 949–63, https://doi.org/10.1038/s41573-019-0047-y.

12. Philipp M. Cromm and Craig M. Crews, "Targeted Protein Degradation: from Chemical Biology to Drug Discovery," *Cell Chem. Biol.* 24, no. 9 (Sept. 21, 2017): 1181–90, https://doi.org/10.1016/j.chembiol.2017.05.024.
13. Philip P. Chamberlain et al., "Evolution of Cereblon-Mediated Protein Degradation as a Therapeutic Modality," *ACS Med. Chem. Lett.* 10, no.12 (Nov. 12, 2019): 1592–1602, https://pubs.acs.org/doi/10.1021/acsmedchemlett.9b00425.
14. Dhanusha A. Nalawansha and Craig M. Crews, "PROTACs: An Emerging Therapeutic Modality in Precision Medicine," *Cell Chem. Biol.* 27, no. 8 (Aug. 20, 2020): 998–1014, https://doi.org/10.1016/j.chembiol.2020.07.020.
15. Yu Ding, Yiyan Fei, and Boxun Lu, "Emerging New Concepts of Degrader Technologies," *Trends Pharmacol. Sci.* 41, no. 7 (July 2020): 464–74 https://www.cell.com/trends/pharmacological-sciences/pdf/S0165-6147(20)30091-2.pdf.
16. Li Zhaoyang et al., "Allele-Selective Lowering of Mutant HTT Protein by HTT–LC3 Linker Compounds," *Nature* 575 (Nov. 7, 2019): 203–09, https://www.nature.com/articles/s41586-019-1722-1.
17. Eric Valeur et al., "New Modalities for Challenging Targets in Drug Discovery," *Angew. Chem. Int. Ed.* 56, no. 35 (Aug. 21, 2017): 10294–323, https://doi.org/10.1002/anie.201611914.

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