Cell Research

www.nature.com/cr
www.cell-research.com

RESEARCH HIGHLIGHT

An engineered surrogate poly(A) tail to wag translation initiation

Stefan Lohse[1] and Wilfried Weber[1,2,3]

© The Author(s) under exclusive licence to Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences 2023

Cell Research (2024) 34:95–96; https://doi.org/10.1038/s41422-023-00903-2

A novel approach for controlling translation initiation in mammalian cells is demonstrated based on the conditional attachment of eukaryotic translation initiation factor-binding proteins to the 3′ UTR of mRNAs via small molecule-, light-, or protein-responsive interactions. The technology overcomes limitations of previously used transcription-based switches and was shown to be functional in managing diabetes or tumor growth in preclinical animal models.

Technologies to precisely control gene expression and protein biosynthesis in mammalian cells represent a cornerstone technology in synthetic biology and advanced gene-based medicine. The on-command induction of therapeutic transgenes would allow the disease-tailored production of therapeutic proteins directly in the patient.[1, 2] Most available technologies rely on the control of gene expression by activating or reconstituting transcription factors in response to exogenous or endogenous small molecules or to light.[1–3] The potential of such approaches has been broadly demonstrated in preclinical animal models targeting a variety of diseases and is currently on the way towards clinical application.[4]

Whereas control at the level of gene transcription is highly efficient for many applications, it inherently comes with the drawback of slow reaction kinetics as sufficient levels of mRNA must first be synthesized before translation can occur without affecting overall protein synthesis efficacy. Similarly, the off-kinetics of transcription-based switches are slow. Although mRNA synthesis might be stopped immediately, protein biosynthesis will occur until the mRNA pools are degraded. To overcome these limitations, Shao et al.[5] developed a novel approach for controlling protein biosynthesis directly at the level of translation initiation.

Eukaryotic translation is a tightly controlled process governed by stepwise formation of protein complexes.[6] During initiation, the target mRNA is unfolded to allow scanning and identification of the initiation codon. The scanning protein complex refolds the mRNA into a closed conformation in which the 3′ poly(A) tail forms a closed loop with the 5′ cap region bridged by translation initiation and poly(A) tail binding proteins[6] (Fig. 1). The design of synthetic mRNA motifs and translation initiation factors that interact with the host cell's translation machinery mimicking the closed-loop refolding would offer the prospect of a controlled protein biosynthesis system reducing unwanted off-target effects and metabolic overload of biosynthesis.

Shao et al. developed such an approach by replacing natural poly(A) tails with synthetic RNA-binding protein (RBP) motifs (aptamers) and expression of synthetic translation initiation factors (STIFs) in a genetically encoded manner (Fig. 1). The combination of a poly(A) tail-specific shRNA or a self-cleaving ribozyme and multiple tandem aptamer repeats resulted in reduced background expression of the protein of interest, a strict STIF dependency, and improved mRNA stability. STIF-dependent translation required interaction with endogenous translation initiation factors providing further evidence for the closed-loop model of eukaryotic translation. Moreover, tethering the eukaryotic translation initiation factor eIF4G in the closed-loop is essential for efficient target mRNA translation but does not require a natural poly(A) tail. This is in analogy to somatic cell histone mRNAs that naturally lack a poly(A) tail and are efficiently translated without competing with the polyadenylated mRNA pool.[6]

The on-demand production and release of drugs by eukaryotic cells requires the implementation of stimulus-specific platforms that enable the detection of mechanical, physical, optical, and chemical stimuli such as temperature, light or compounds used in cancer or antiviral therapy.[1–3] Shao et al. modified their RBP aptamer STIF system by incorporating dimerization elements responsive to compounds or light to enable trigger-induced dimerization of split STIFs. In addition, intracellular signaling, such as the strong mitogen-activated protein kinase signaling that occurs in cancer cells, or specific oncoproteins, have been functionalized as a genetically encoded sensing platform to enable specific drug release in appropriately activated malignant cells. Eventually this can convert malignant cells into biocomputing sensors with built-in self-destruction. The invented platform could serve as a broadly applicable framework for providing individualized solutions to (even complex) medical applications, and the authors provided proof of concepts in animal models. In one of these cases, the authors created a small molecule-dependent gene circuit using the FDA-approved antiviral drug grazoprevir. The drug can be used off-label and is characterized by its bioavailability and tolerability. The synthetic mRNA contained tandem repeats specific for a STIF variant, which consist of two separate components that assemble in the presence of grazoprevir and enable trigger-specific induction of insulin expression in a diabetes model in mice. Finally, they demonstrated the potential of their approach in a mouse cancer model by xenotransplantation of genetically engineered mouse cancer cells and in vivo transfection with plasmids encoding a translational sensor. This

[1]INM-Leibniz Institute for New Materials, Campus D2 2, 66123 Saarbrücken, Germany. [2]Department of Materials Science and Engineering, Saarland University, Campus D2 2, 66123 Saarbrücken, Germany. [3]CIBSS-Centre for Integrative Biological Signalling Studies, University of Freiburg, Schänzlestr. 18, 79104 Freiburg, Germany.
email: Wilfried.weber@leibniz-inm.de

Published online: 4 January 2024

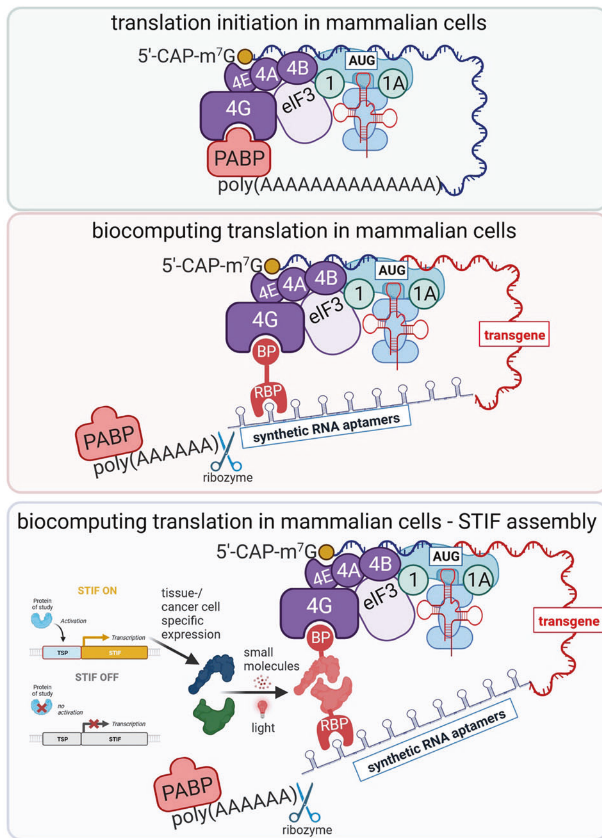

Fig. 1 Schematic illustration of translation initiation in mammalian cells. In the closed-loop model, the target mRNA is refolded by eukaryotic initiation factors (eIF proteins) and the poly(A)-tail binding protein (PABP). Based on this mechanism, Shao et al. developed a synthetic translation initiation technology that uses RNA aptamer repeats instead of the native poly(A) tail, which is removed from the 3′-end by custom-designed shRNAs or ribozymes, allowing expression of any transgene of interest to exclusively depend on a STIF that consists of an exogenous RBP domain and an initiation factor 4G binding protein (BP) domain. By integrating elements that respond to small molecules, light or activated signaling pathways, and by splitting the STIF into separately controlled proteins genetically controlled by tissue-specific promoters (TSPs) that require efficient dimerization for translation initiation, the system can be adapted to integrate different sense-and-response platforms according to the requirements of each specific medical application.

sensor platform comprised two levels of specificity, which were fulfilled by using a TSP for STIF expression and a target-specific (STIF) sensor for the induction of target protein expression. Ultimately, this led to tumor cell-specific expression of the pro-apoptotic protein BAX and thus ultimately to self-sufficient killing of cancer cells.

Prior to clinical translation, there are some challenges to be addressed. For example, the synthetic proteins involved in the system could be perceived by immune cells. Balancing highly flexible sense-and-response biocomputing platforms without implementing potent neoepitopes is to be achieved towards clinical translation.[7, 8] The use of human-derived RNA-binding and dimerization domains might overcome these issues. Further, the expression level of the elongation factor-binding proteins designed in this study might need to be carefully controlled to avoid interference with physiological translation initiation in the cell. The complexity of the system may require the application of multiple separate viral vectors to permanently install the gene switch in the patient and enable tissue- and context-specific drug release on demand with a long-term perspective. In addition, the protein sensor-based platforms require a true cancer-specific oncoprotein that is exclusively detected in cancer cells or a concentration-dependent detection of a protein that is specifically overexpressed in cancer cells under certain conditions, such as the p16^{INK4A} marker in human papillomavirus-associated entities.

Beyond applications in synthetic biology and gene-based therapy, the newly developed synthetic platform has the potential to serve as a toolbox to answer still unresolved questions in translation in mammalian cells. For example, the individual RNA aptamer STIF platform variants each required an exact number of tandem repeats. Considering the still debated necessity of poly(A) tail length for sufficient translation,[6] this side result is worth further investigation.

The poly(A) surrogate technology presented here is highly versatile to convert the presence of small molecules, proteins, or optical signals into translation initiation. It fills an important gap in the available technologies to control mammalian cell fate and function with broad applications beyond gene-based therapy. For example, such technology might be used to add an additional control layer to biocomputing platforms that might be used in advanced sensing systems. Also, given the conserved mechanisms of translation initiation among eukaryotes, this approach might be transferred to yeasts or plants opening broad applications in biosensing, bioproduction or agricultural technology.

REFERENCES

1. Bojar, D., Scheller, L., Hamri, G. C., Xie, M. & Fussenegger, M. *Nat. Commun.* **9**, 2318 (2018).
2. Maity, D., Guha Ray, P., Buchmann, P., Mansouri, M. & Fussenegger, M. *Adv. Mater.* **35**, e2300890 (2023).
3. Kolar, K., Knobloch, C., Stork, H., Žnidarič, M. & Weber, W. *ACS Synth. Biol.* **7**, 1825–1828 (2018).
4. Li, H. S. et al. *Science* **378**, 1227–1234 (2022).
5. Shao, J. et al. *Cell Res.* https://doi.org/10.1038/s41422-023-00896-y (2023).
6. Jackson, R. J., Hellen, C. U. & Pestova, T. V. *Nat. Rev. Mol. Cell Biol.* **11**, 113–127 (2010).
7. Balu, R., Dutta, N. K., Dutta, A. K. & Choudhury, N. R. *Nat. Commun.* **12**, 149 (2021).
8. Bueno, E. & Glowacki, J. *Nat. Rev. Rheumatol.* **5**, 685–697 (2009).

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Wilfried Weber.

Reprints and permission information is available at http://www.nature.com/reprints