Regulatory Functions of the Upstream Open Reading Frame in mRNA of the *MCRS1* Human Gene

Baltin S. M.[1]*, Suspitsyna A. D.[2], Shepelev N. M.[3, 4], Rubtsova M. P.[3, 4], Dontsova O. A [2, 3, 4]

[1] *Biological Faculty, Lomonosov Moscow State University, Moscow, Russia*

[2] *Center for Molecular and Cellular Biology, Skolkovo Institute of Science and Technology, Moscow, Russia.*

[3] *Chemistry Faculty, Lomonosov Moscow State University, Moscow, Russia.*

[4] *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia.*

* *s.baltin@rambler.ru*

Key words: small open reading frame; sORF; uORF; mRNA; translation; *MCRS1*

Skoltech
Skolkovo Institute of Science and Technology

ИБХ

Objective of the Study

Introduction: The eukaryotic cell cycle consists of interphase and a mitotic phase. Precise regulation of the cell cycle is necessary for cell proliferation and development of the entire organism. The *MCRS1* gene is one of the important regulators of the cell cycle. Its overexpression is associated with tumorigenesis. The biological functions of the MCRS1 protein are very diverse: it regulates transcription factors necessary for cell proliferation and response to stress, is involved in post-translational modifications of histones, activation of the mTOR pathway, maintenance of centrosome integrity and microtubule dynamics, and more (**Figure 1**).

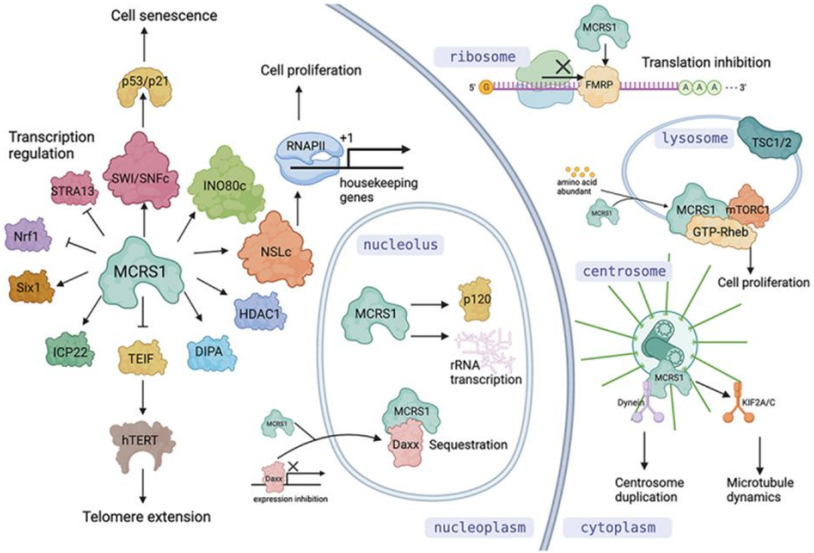

Fig. 1. Functions of the MCRS1 protein and its isoforms in a eukaryotic cell (Huang, Ching-Jung et al., 2023).

Background. The CRISPR-Cas9 screening previously performed in the laboratory has discovered that the disruption of upstream ORF located in *MCRS1* gene affects viability of human cells. Further research focused on the regulation of the expression of this gene, in particular the translation of its mRNA, since this gene performs many important functions in the cell.

We analyzed available ribosome profiling data for the main mRNA isoform using the Trips-viz browser. The number of aggregated and mapped reads in the 5' untranslated region that were higher than in the annotated coding sequence of the main ORF, which indicates uORF translation (**Figure 2**). The *MCRS1* gene mRNA contains three small open reading frames in the 5' untranslated region, varying in length (sORF#1, sORF#2, sORF#3). The aim of the research is to study their biological function.

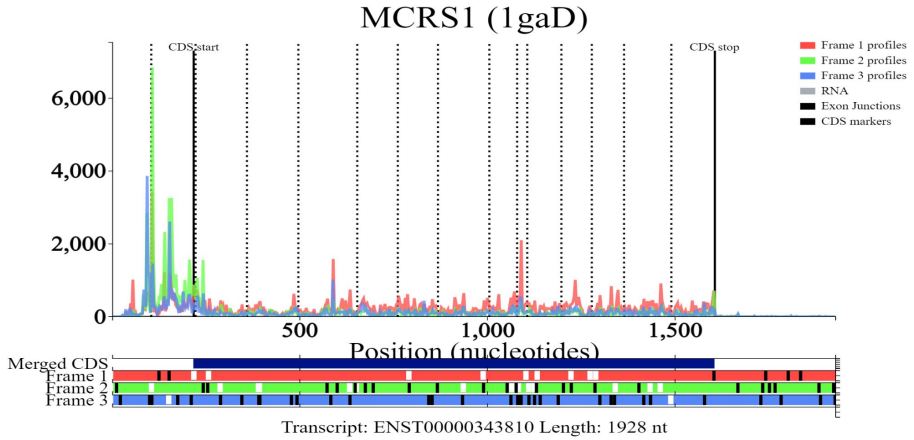

Fig. 2. Ribosome profiling data of *MCRS1* main transcript (Trips-viz browser).

Materials and Methods

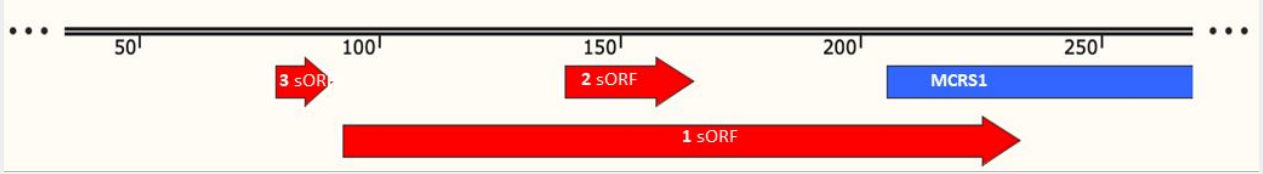

Fig. 3. Relative arrangement of sORFs in the *MCRS1* transcript.

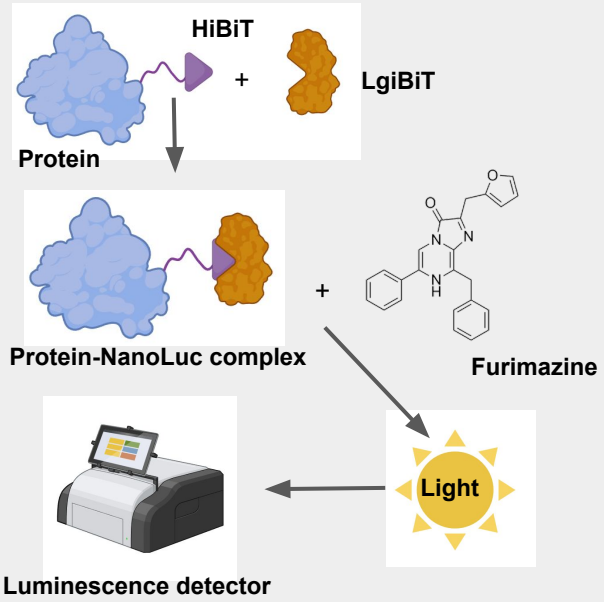

Fig. 5. sORF peptide detection was carried out using endogenous tagging by HiBiT sequence

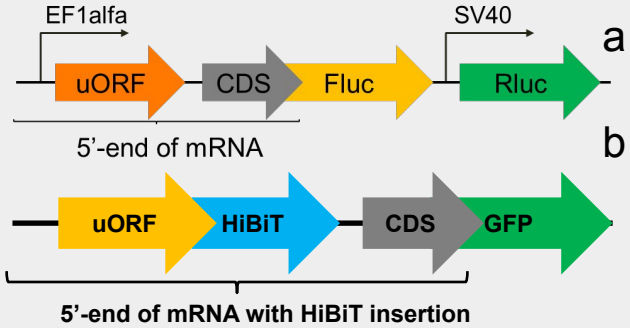

Fig. 4. Dual luciferase reporters were used to study main ORF translation (a). Validation of translation was done using fusion with HiBiT sequence (b). The experiment was conducted on the HEK293T cell line.

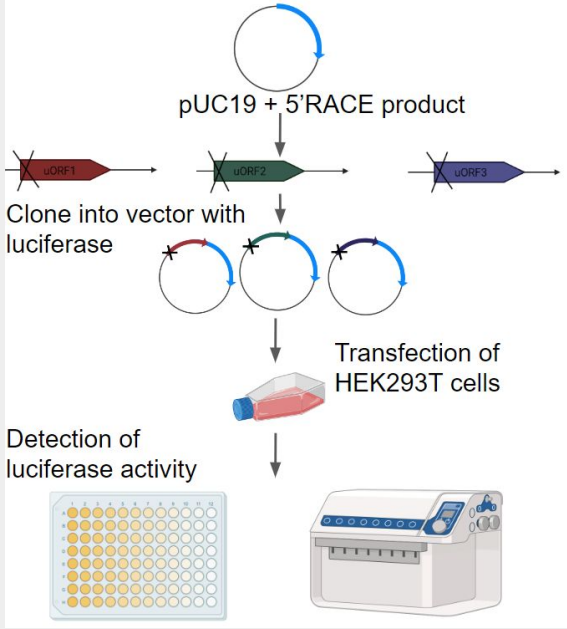

Fig. 6. The scheme of constructing reporters with point mutations and their application.

<u>The experiment pipeline:</u> To study the mechanism translation regulation, we used dual luciferase reporter constructions. Firefly luciferase (Fluc) was coupled with 5'-end of *MCRS1* mRNA including uORFs and a portion of *MCRS1* main coding sequence with its start codons, while *Renilla* luciferase (Rluc) was used as an internal control. As we can measure luciferase activity separately, we are able to understand the influence of the uORF on the main ORF (**Figure 4a**). To validate the translation of *MCRS1* sORFs (**Figure 3**) we used HiBiT tagging (**Figure 4b**) and subsequent detection of the tagged peptides (**Figure 5**).

Results

sORF#1 is translated endogenously

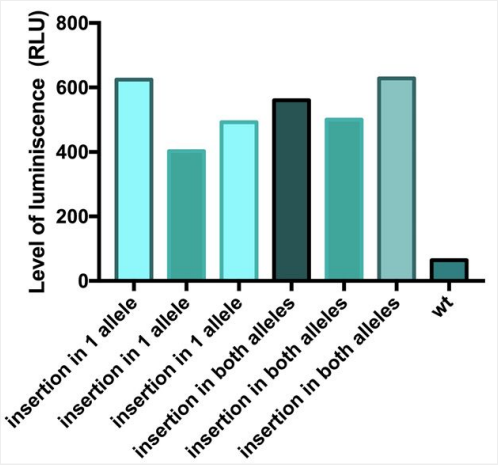

Fig. 7. Level of HiBiT-mediated luminescence for sequenced monoclonal cell lines and wild type cells.

sORF#1 repress *MCRS1* ORF translation

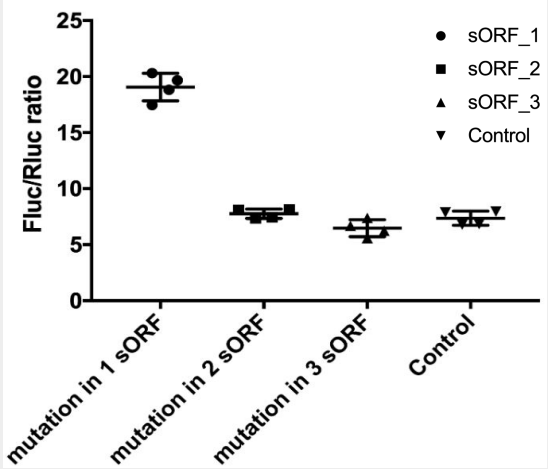

Fig. 8. Relative luciferase activity for different reporter constructs.

sORF#2 can also be translated

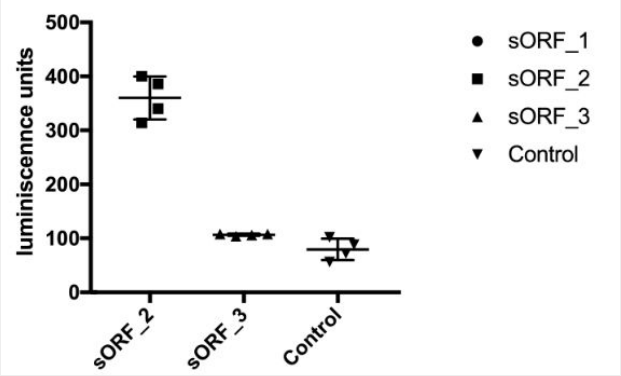

Fig. 9. Level of HiBiT-mediated luminescence of cell lysates after transfection using reporter constructs with HiBiT tagged sORFs.

sORF#1 was labeled by HiBiT sequence using CRISPR-Cas9 system. Selected monoclonal cell lines have higher HiBiT-mediated luminescence indicating the presence of the tagged peptide in cells (**Figure 7**).
Mutation in the start codon of sORF#1 increases the translation of the luciferase reporter by approximately 2.6-fold (**Figure 8**).

This experiment shows the importance of the 2nd start codon (**b**) but not the 1st start codon of sORF#1 for the main ORF translation. 2nd start codon is not involved in translation reinitiation (**c**). Truncation of the sORF #1 activates the translation of *MCRS1* mRNA (**d-e**), and its fusion with the main *MCRS1* ORF does not affect translation (**f**).

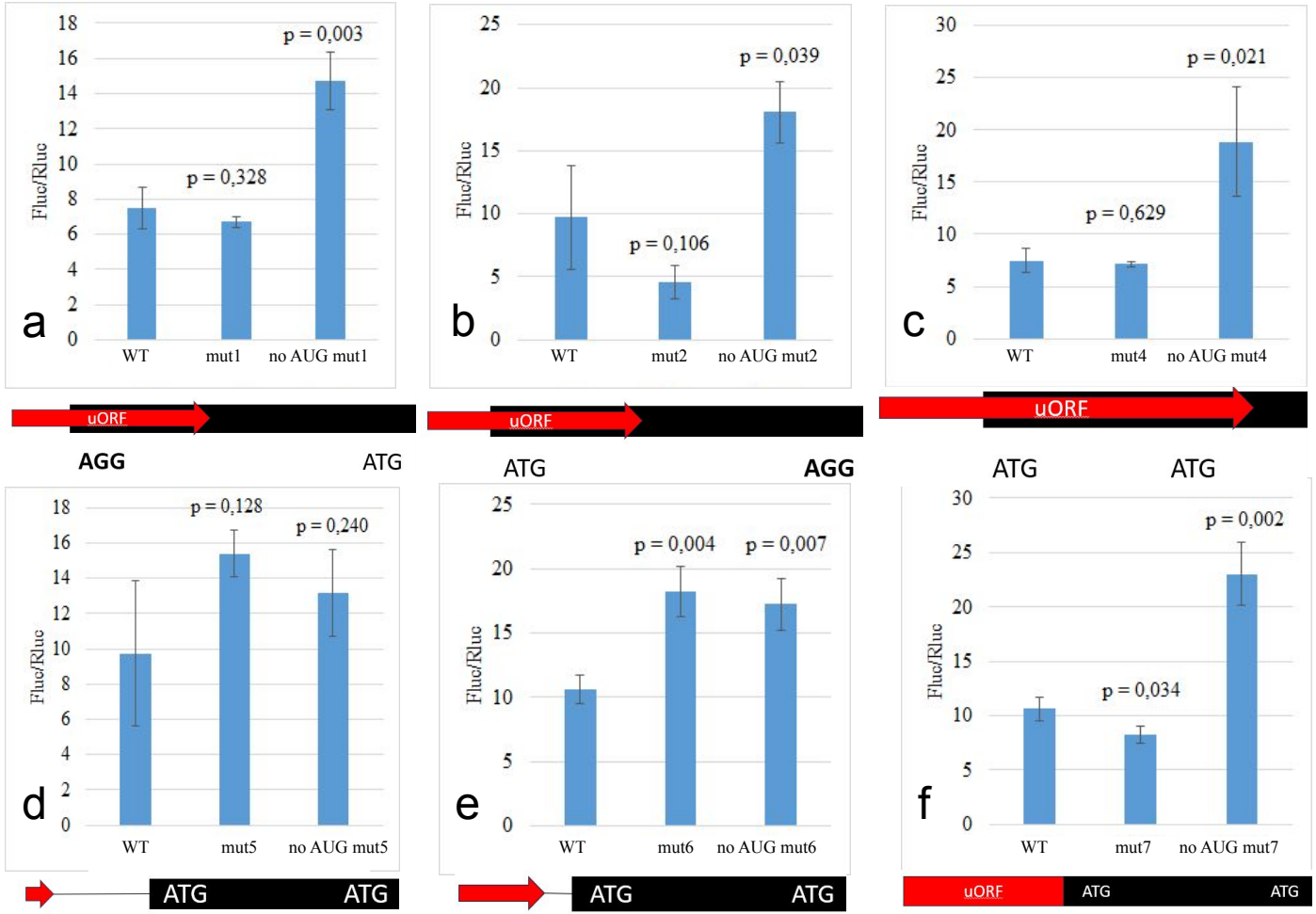

Fig. 10. Relative luciferase activity upon mutations in the *MCRS1* in dual luciferase reporter. WT – wild type, **mut№** – sORF#1 is translated and it has point mutation, **no AUG mut№** – small ORF is not translated and it has the same mutation. The columns of the graph show the average values of the Fluc/Rluc activity ratios. The whiskers show standard deviations. Below the graphs the impact of mutations on sORF#1 and main ORFs is schematically shown (**Figure 10**). p-values are calculated using Student's t-test.

Conclusions

1. The sORF #1 of the *MCRS1* gene is translated and has an influence on cell viability.
2. The sORF #2 of the *MCRS1* gene can also be translated.
3. The sORF #1 of the *MCRS1* gene represses the translation of the main ORF.
4. The second start codon of the *MCRS1* main ORF is involved in translation initiation and is sufficient for translation of *MCRS1* in the absence of the first start codon. The second start codon is not involved in translation reinitiation.
5. Truncation of the sORF #1 activates the translation of *MCRS1* mRNA, and its fusion with the main *MCRS1* ORF does not affect translation.