In-Cell Discovery of RNA–Protein Interactions

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TWO-SECTION SUMMARY

Numerous methods have been developed to define proteins bound to an RNA of interest [1-6]. Existing techniques commonly rely on probe-based RNA capture and identification of co-purified proteins by mass spectrometry, which often present limitations associated with crosslinking, RNA enrichment, and protein detection. The incPRINT (in cell protein-RNA interaction) method overcomes these challenges by using stably expressed recombinant luciferase tethered to an MS2-tagged RNA of interest through MS2 coat protein (MS2CP) [7]. If an interaction between a test RNA and a test protein occurs in the cell, it is measured by luminescence upon cell lysis and immunoprecipitation of the tagged protein.

To identify RNA-bound proteomes, high-throughput immunoprecipitation of thousands of tagged proteins [8-10] is carried out, followed by luminescence-based detection of their interactions with an individual RNA. The approach is suitable for discovery of proteins interacting with full-length transcripts or with specific RNA regions.

ADVANTAGES:

- Does not rely on direct RNA pulldowns, thus circumventing a step often plagued by low efficiency.
- Avoids crosslinking and the associated inefficiency and biases.
- Not limited by low expression levels of test RNA or proteins.
- Can identify proteomes of specific RNA regions and provide RNA-region-specific resolution of protein binding.
- Can identify transient RNA-protein interactions.
- One cell line is used for all applications.

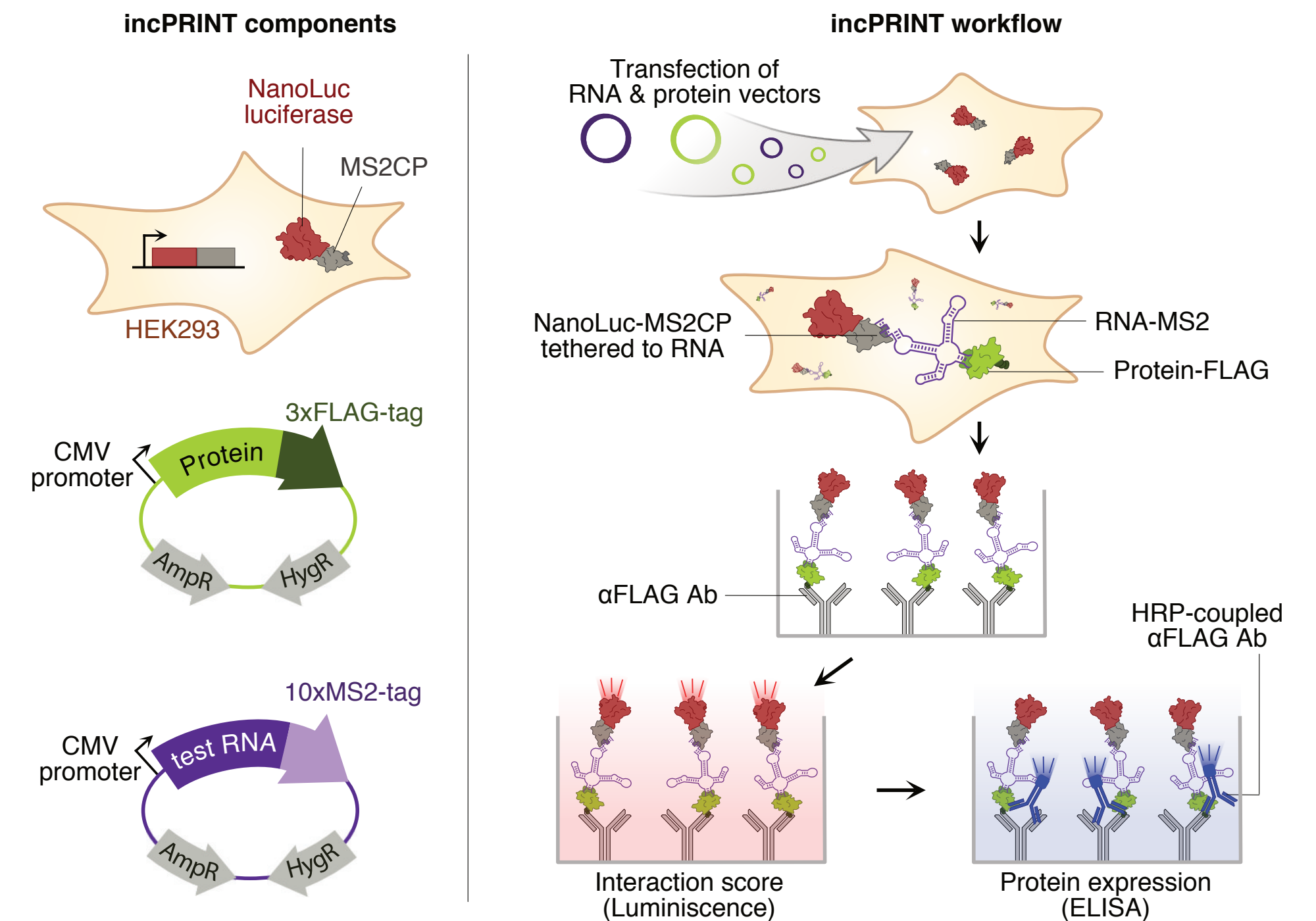
- The protein library is not limited in size and can be expanded or reduced depending on experimental needs.
- Flexibility of throughput ranging from high to low allows usage of incPRINT as an orthogonal method for validation of individual RNA-protein interactions.
- Quantitative luciferase enables structure-function analyses and analyses of mutated or disordered RNA-protein interactions.

CHALLENGES:

- incPRINT is a primary binding assay that does not consider the developmental context or timing of RNA-protein interactions.
- A subset of proteins may interact with MS2 stem loops but not with the test RNA.
- Protein tags may alter protein expression or binding.
- Some interactions may be indirect; additional validation assays are required to establish direct RNA binding.
- Some RNA-protein interactions may require additional factors that are not expressed in the cell line used for the assay.
- Ectopic expression may affect sub-cellular localization of test RNAs and proteins.

LITERATURE

1. Chu, C. et al. (2015) Systematic discovery of Xist RNA binding proteins. Cell 161 (2), 404-16.
2. McHugh, C.A. et al. (2015) The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3. Nature 521 (7551), 232-6.
3. Ramanathan, M. et al. (2018) RNA-protein interaction detection in living cells. Nat Methods 15 (3), 207-212.
4. Lee, H.Y. et al. (2013) RNA-protein analysis using a conditional CRISPR nuclease. Proc Natl Acad Sci U S A 110 (14), 5416-21.
5. Zielinski, J. et al. (2006) In vivo identification of ribonucleoprotein-RNA interactions. Proc Natl Acad Sci U S A 103 (5), 1557-62.
6. Slobodin, B. and Gerst, J.E. (2010) A novel mRNA affinity purification technique for the identification of interacting proteins and transcripts in ribonucleoprotein complexes. RNA 16 (11), 2277-90.
7. Graindorge, A. et al. (2019) In-cell identification and measurement of RNA-protein interactions. Nat Commun 10 (1), 5317.
8. Baltz, A.G. et al. (2012) The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. Mol Cell 46 (5), 674-90.
9. Castello, A. et al. (2012) Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. Cell 149 (6), 1393-406.
10. Taipale, M. et al. (2012) Quantitative analysis of HSP90-client interactions reveals principles of substrate recognition. Cell 150 (5), 987-1001.

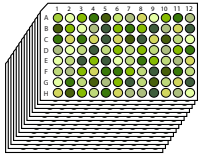

incPRINT components
NanoLuc luciferase
MS2CP
HEK293
3xFLAG-tag
CMV promoter
Protein
AmpR
HygR
10xMS2-tag
CMV promoter
test RNA
AmpR
HygR
incPRINT workflow
Transfection of RNA & protein vectors
NanoLuc-MS2CP tethered to RNA
RNA-MS2
Protein-FLAG
αFLAG Ab
HRP-coupled αFLAG Ab
Interaction score (Luminiscence)
Protein expression (ELISA)

Library of human FLAG-tagged proteins

High-throughput identification of interactors

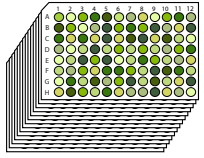
Data analysis and normalization

~1500 RNA-binding proteins

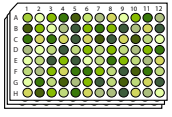


Protein X
Protein Y
Protein Z
...

~1300 transcription factors



~170 chromatin-associated proteins



Interaction

No interaction