Neurosci. Bull. December, 2017, 33(6):763–765
https://doi.org/10.1007/s12264-017-0161-y

www.neurosci.cn
www.springer.com/12264

RESEARCH HIGHLIGHT

MEMOIR: A Novel System for Neural Lineage Tracing

Zhifu Wang[1,2,3] · Jianhong Zhu[1,2,3]

Received: 24 January 2017 / Accepted: 11 May 2017 / Published online: 5 August 2017

Memory by Engineered Mutagenesis with Optical *In situ* Readout (MEMOIR) is a novel strategy for lineage tracing that combines Cas9/gRNA and sequential multiplexed single-molecule RNA fluorescence hybridization (seqFISH) [1], which was created by Cai Long *et al.* at the California Institute of Technology [2]. In MEMOIR, dynamic cellular event histories are recorded, then read out in single cells using seqFISH. Here, we introduce the principles and the implementation processes of the MEMOIR system (Fig. 1), and further discuss its merits for neural lineage tracing compared with classical strategies.

In principle, two powerful techniques are essential for MEMOIR: first, a set of genomic elements termed 'barcoded scratchpad', whose sequences can be specifically targeted and deleted by Cas9-gRNA, are integrated into the genome of cells. Therefore, for a single cell, its progeny inherit the alterations of the barcoded scratchpads, and all the daughter cells can be traced permanently. Second, seqFISH supports *in situ* readout of the states of barcoded scratchpads in single cells. By analyzing the distribution patterns of these mutations, the lineage relationships of targeted cells are defined. For instance, whether cells are sisters or cousins have been identified accurately. It has been verified that seqFISH can distinguish the readouts of 13 barcoded scratchpads in a single cell after growth for 3–4 divisions [2].

Clearly, the Cas9/gRNA-targeted 'barcoded scratchpad' and seqFISH are the keys for the MEMOIR system to record and readout lineage information. A barcoded scratchpad is a bipartite genetic recording element (i.e. a scratchpad element and a barcode element). Ten repeat units constitute the scratchpad element, which can be targeted by gRNA. Accordingly, the components of Cas9 and gRNA stochastically lead scratchpad to double-strand breaks, namely "collapse". In order to modulate Cas9 activity externally, an inducible degron is contained in a variant Cas9. To distinguish different scratchpads, a unique barcode, as a diacritical sign, is adjoined with each scratchpad. The bipartite element is co-transcribed, and its mRNAs are detected by seqFISH. There are two steps in this novel system: (1) Cas9 [3, 4] stochastically breaks these genetic barcoded-scratchpads and accumulates collapses with each cell division, and the states of barcoded scratchpads reflect the relationships between different daughter cells; and (2) seqFISH is used to interrogate the states of barcoded scratchpads *in situ*. Based on the readout of seqFISH, a lineage tree is reconstructed within the native context.

Another crucial technique is seqFISH, developed by the Cai Long group. They presented a scheme of sequential rounds of hybridization to multiplex mRNAs in a single cell. This differs from FISH in that, after hybridizing and imaging probes, these probes are removed by DNase I and the fluorophores are bleached. Then a new round of hybridization is implemented in the same cell with another series of barcode probes. They have verified that the method maintains 77.9% ± 5.6% of co-localization after 3

✉ Jianhong Zhu
jzhu@fudan.edu.cn

[1] Department of Neurosurgery, Fudan University Huashan Hospital, Shanghai 200040, China

[2] National Key Laboratory of Medical Neurobiology, The Institutes of Brain Science and The Collaborative Innovation Center for Brain Science, Shanghai Medical College, Fudan University, Shanghai 200040, China

[3] Translational Medical Center for Stem Cell Therapy, Shanghai East Hospital, Shanghai 200120, China

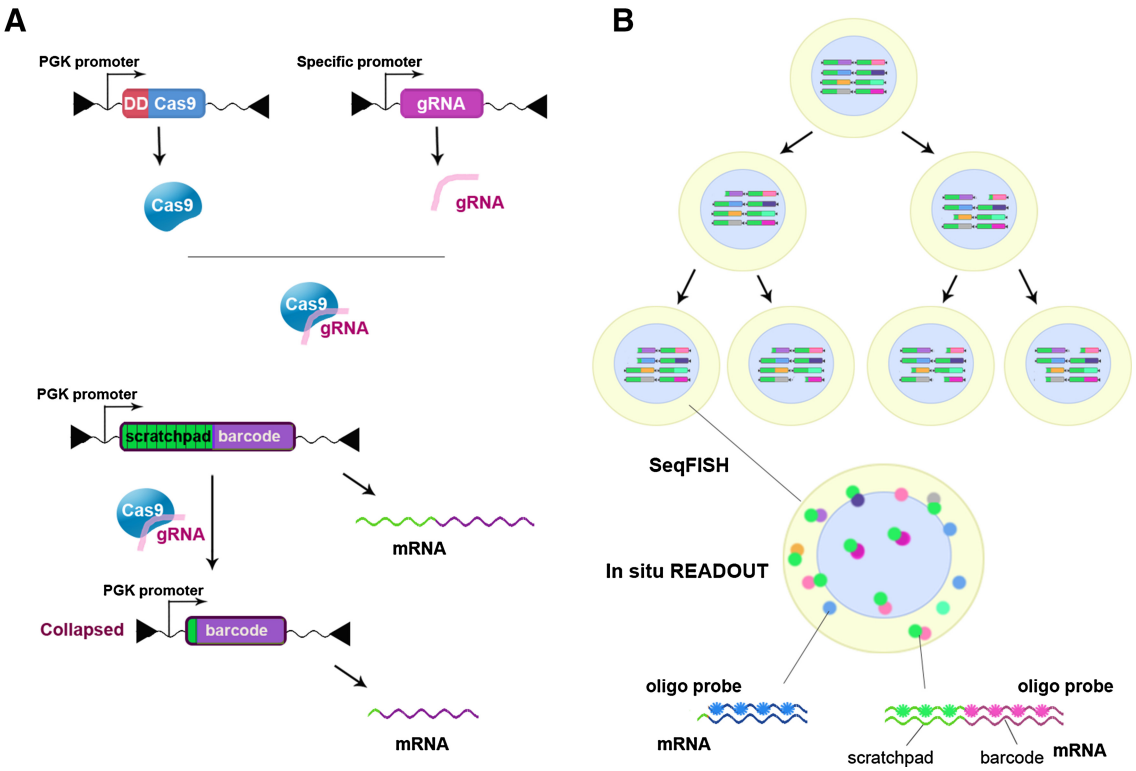

Fig. 1 Principle of MEMOIR system. **A** In MEMOIR system, three types of elements are integrated into the genome, including a Cas9 variant, a set of barcoded scratchpads and a specifically inducible gRNA targeting the scratchpad. Barcoded scratchpads can be collapsed irreversibly by Cas9/gRNA. An inducible degron modulates Cas9 activity. Inverted black triangles in (**A**) indicate piggyBac terminal repeats. **B** Schematic of the MEMOIR working in cells: During cell proliferation, stochastic collapsed scratchpads accumulate gradually. Subsequently, single mRNA molecules can be tested with scratchpad-specific probes (green, inset), and a set of barcode-specific probes (purple, pink, inset) through sequential circles of hybridization and imaging.

rounds of hybridization. In a recent article, they showed how to use this technique to detect the expression levels of 249 genes in mouse hippocampus [5]. This method allows researchers to analyze the gene activity of cells without separating them from the native context.

Genetic tools have been used for lineage tracing since the early 1990s. Especially, abundant information of cell fate-map has been revealed through the Cre-loxP system in brain and spinal cord. For example, nestin-CreER^{tm}4 lineage-tracing demonstrates that Thbs4^{hi} neural stem cells (NSCs) in the subventricular zone contribute to post-injury astrogenesis [6], corresponding with the protective process in human spinal cord injury [7]. How to reveal and change the fate of NSCs in brain diseases remains a major challenge [8, 9]. However, because of their heterogeneous properties, it is difficult to define a subpopulation with one protein marker. In particular, brainbow has been used to reveal NSC heterogeneity, but the readout merely represents the original and terminal states, whereas neural development or regeneration is dynamic. In MEMOIR, not only are the relationships of all daughter cells identified, but the relationships of parent cells can be calculated based on the states of barcoded scratchpads. Therefore, a lineage tree is reconstructed and the directions of NSC differentiation are revealed. In addition, many lineage-tracing strategies refer to the barcode gene, especially a method termed genome editing of synthetic target arrays for lineage tracing (GESTALT) [10]. A substantial difference is that MEMOIR obtains the readout through seqFISH instead of single-cell RNA sequencing (RNA-seq). Compared with RNA-seq, seqFISH only measures the transcription levels of 100–200 genes, but with much greater quantitative power [5]. And seqFISH can also provide multiplex mRNA imaging. In conclusion, the MEMOIR system indicates a novel direction for lineage tracing. As a powerful tool for investigating the fate of adult somatic stem/progenitor cells in the native context, it plays a complementary role with the classical strategies in lineage tracing.

Acknowledgements Research work in the Zhu lab was supported by the grants from the National Natural Science Foundation of China (81271003) and the Ministry of Science and Technology of China (2013CB967400, 2012CB966300, and ZJ2014-ZD-002).

References

1. Lubeck E, Coskun AF, Zhiyentayev T, Ahmad M, Cai L. Single-cell in situ RNA profiling by sequential hybridization. Nat Methods 2014, 11: 360–361.
2. Frieda KL, Linton JM, Hormoz S, Choi J, Chow KK, Singer ZS, *et al.* Synthetic recording and in situ readout of lineage information in single cells. Nature 2017, 541: 107–111.
3. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, *et al.* Multiplex genome engineering using CRISPR/Cas systems. Science 2013, 339: 819–823.
4. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012, 337: 816–821.
5. Shah S, Lubeck E, Zhou W, Cai L. *In situ* transcription profiling of single cells reveals spatial organization of cells in the mouse hippocampus. Neuron 2016, 92: 342–357.
6. Benner EJ, Luciano D, Jo R, Abdi K, Paez-Gonzalez P, Sheng H, *et al.* Protective astrogenesis from the SVZ niche after injury is controlled by Notch modulator Thbs4. Nature 2013, 497: 369–373.
7. Zhu T, Tang Q, Gao H, Shen Y, Chen L, Zhu J. Current status of cell-mediated regenerative therapies for human spinal cord injury. Neurosci Bull 2014, 30: 671–682.
8. Pan X, Chen Z, Fei G, Pan S, Bao W, Ren S, *et al.* Long-term cognitive improvement after benfotiamine administration in patients with Alzheimer's disease. Neurosci Bull 2016, 32: 591–596.
9. Chen L, Li C, Zhai J, Wang A, Song Q, Liu Y, *et al.* Altered resting-state signals in patients with acute stroke in or under the thalamus. Neurosci Bull 2016, 32: 585–590.
10. McKenna A, Findlay GM, Gagnon JA, Horwitz MS, Schier AF, Shendure J. Whole-organism lineage tracing by combinatorial and cumulative genome editing. Science 2016, 353: aaf7907.