

during ageing and this interferes with proteostasis and removal of aggregated proteins in aged qNSCs. Autophagic flux and protein aggregate clearance were restored by subjecting mice to fasting or by expressing constitutively active TFEB in aged qNSCs. TFEB expression also enhanced activation of aged qNSCs, indicating that deterioration of NSC function during ageing can be restored by stimulating the autophagy–lysosome system.

In sum, this study revealed differential proteostasis control in qNSCs and aNSCs and that lysosomal function is important to maintain NSC activity during ageing. It will be interesting to explore whether similar mechanisms operate in other types of adult stem cells and whether lysosomal activity can be enhanced, for example by controlled fasting regimes, to counteract stem cell ageing.

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ORIGINAL ARTICLE Leeman, D. S. et al. Lysosome activation clears aggregates and enhances quiescent neural stem cell activation during aging. *Science* **359**, 1277–1283 (2018)

NHEJ is crucial for immunoglobulin class-switch recombination (CSR), and the RINN proteins were necessary for CSR *in vitro*. The RINN proteins were required for restricting end resection, thereby explaining the necessity of shieldin for NHEJ. Finally, 53BP1 or REV7 depletion provides resistance to PARP inhibitors (PARPi) in BRCA1-deficient cancer cells by restoring HDR. Likewise, shieldin depletion restored viability to PARPi-treated BRCA1-deficient cells.

The data indicate that by shielding DSBs from resection, shieldin functions as an effector of 53BP1 in DSB-repair pathway choice. It remains to be seen how shieldin is connected to RIF1 and 53BP1 and how it shields DSBs, and it would be important to study whether shieldin deficiency provides resistance to PARPi in clinical settings.

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ORIGINAL ARTICLE Gupta, R. et al. DNA repair network analysis reveals shieldin as a key regulator of NHEJ and PARP inhibitor sensitivity. *Cell* <https://doi.org/10.1016/j.cell.2018.03.050> (2018)

FURTHER READING Chang, H.H.Y. et al. Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat. Rev. Mol. Cell Biol.* **18**, 495–506 (2017)



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Regulation of gene expression relies on various molecular mechanisms, which affect different stages of mRNA biogenesis. In animals, microRNAs (miRNAs) suppress gene expression by base-pairing with target mRNAs through partial sequence complementarity, which results in mRNA deadenylation and decay and in translation inhibition. These processes are mediated by Argonaute proteins and associated factors such as trinucleotide repeat-containing gene 6B protein (TNRC6B), which together with miRNAs constitute the miRNA-induced silencing complex (miRISC). How the structure and organization of miRISC modulates its function is unknown. Sheu-Gruttadauria and MacRae now show that binding of Ago2 to TNRC6B promotes phase separation and the formation of miRISC-containing liquid droplets both *in vitro* and in cells, which can increase the functionality of miRISC.

Structural data of purified human Ago2 and TNRC6B identified three tryptophan (Trp)-binding pockets in the PIWI domain of Ago2, of which two were previously reported, and revealed they are arranged at equal distances from each other on the surface of Ago2. Biochemical binding studies showed that all three pockets can recognize multiple distinct Trp residues in the glycine–tryptophan (GW)-rich N-terminal domain of TNRC6B. This suggested that the interactions between Ago2 and TNRC6B are complex and that the underlying structure of miRISC is heterogeneous at the atomic level.

In vitro, a solution of TNRC6B became turbid with the introduction of purified Ago 2; microscopic, phase-separated liquid droplets were formed in dependence on Ago2 concentration. Moreover, fluorescence recovery after photobleaching experiments using

GFP–TNRC6B and mCherry–Ago2 indicated that, also in cells, phase separation drives the formation of viscoelastic TNRC6B–Ago2 droplets.

Importantly, in the separated phase, catalytically active Ago2 bound to miRNAs that specifically sequestered target mRNAs from the surrounding solution, and cleaved them. Furthermore, in the presence of Ago2, TNRC6B was able to recruit and concentrate components of the miRISC complex, including components of the CCR4–NOT complex, from soluble lysates.

To assess whether miRISC-associated gene silencing processes remain active in the identified droplets, the authors examined mRNA deadenylation. Using 5′ cap-radiolabeled target mRNA, they found that the rate of poly(A)-dependent 3′ end shortening increased tenfold after adding higher amounts of TNRC6B to induce miRISC condensation. The increase in deadenylation was 100-fold when the reaction was performed with the molecular crowding agent polyethylene glycol to enhance miRISC phase separation.

In summary, human miRISC undergoes phase separation through the formation of multivalent interactions between Ago2 and the GW-rich sequence of TNRC6B, which helps sequester target mRNAs and promotes their deadenylation. Thus, the capacity to form multivalent interactions could have a role in the regulation of miRNA-mediated gene silencing.

Anne Mirabella,
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ORIGINAL ARTICLE Sheu-Gruttadauria, J. & MacRae, I.J. Phase transitions in the assembly and function of human miRISC. *Cell* <https://doi.org/10.1016/j.cell.2018.02.051> (2018)

FURTHER READING Banani, S. F. et al. Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* **18**, 285–298 (2017)