both ATP production and oxygen consumption, confirming the role of these proteins in young and adult worms. No increase in the number of mitochondria was found, but *baz-2* and *set-6* knockouts exhibited activation of the mitochondrial unfolded protein response as a result of increased expression of a set of nuclear genes encoding mitochondrial proteins.

Expression of the mammalian homologues BAZ2B and EHMT1 increased with age in the prefrontal cortex of human brains, and binding to nuclear genes encoding mitochondrial proteins could be observed in HEK293T cells. In mouse primary neuron cultures, *Baz2b* or *Ehmt1* downregulation led to increases in mitochondrial protein expression, ATP production and oxygen consumption.

Adult BAZ2B-deficient mice showed improved brain mitochondrial function, with males also exhibiting decreased age-related weight gain, which suggests effects of BAZ2B on energy metabolism. Cognitive behaviour in male mice aged >18 months showed some effects, with BAZ2B-deficient mice exhibiting improved spatial learning and memory but no changes to exploratory or locomotor behaviour.

Analysis of existing human data revealed a positive correlation between BAZ2B and EHMT1 expression in the prefrontal cortex and progression of Alzheimer disease. A negative correlation with the expression of mitochondrial proteins was also observed, which could mean that increased expression of BAZ2B and EHMT1 with age promotes mitochondrial impairments in Alzheimer disease.

Taken together, the findings suggest that BAZ2B and EHMT1 act as negative regulators of healthy ageing by restricting mitochondrial function through regulation of H3K9 methylation at promoters of target genes. Future studies will need to determine whether targeting these repressors is a viable strategy to prolong healthspan.

Linda Koch

ORIGINAL ARTICLE Yuan, J. et al. Two conserved epigenetic regulators prevent healthy ageing. *Nature* **579**, 118–122 (2020)

Credit: P. Morgan/ Springer Nature Limited

capable of identifying pathogenic and non-pathogenic enhancer variants.

The authors went on to use enSERT to screen mutations in the ZRS enhancer for changes in enhancer function. To this end, they designed non-overlapping constructs with alterations in either 2% or 5% of the enhancer sequence (16 bp or 40 bp, respectively). The 17 tested mutant constructs with 5% changes all reduced or abolished ZRS activity. However, three of the 2% mutant constructs caused gain of enhancer activity, as seen by LacZ expression in the anterior limb buds of transgenic mouse embryos. These variants could include mutations linked to polydactyly in humans that are as yet undetected. To interrogate which specific point mutations contributed to gain of enhancer activity, the authors picked one of the constructs that induced ectopic ZRS activation and introduced each of the 16 single-base pair variants from this construct into the ZRS enhancer individually, testing the effect of each point mutation using enSERT. They found only two single-base pair variants were individually sufficient for ectopic expression of ZRS. These variants were close to the site of a previously characterized human mutation associated with polydactyly. All three polymorphisms were related to the same SOX binding site, potentially implicating these sites in the development of polydactyly and showing the utility of this technique in identifying mechanistic aspects of non-coding DNA-related diseases.

Taken together, these findings suggest enSERT is a scalable technique for screening the involvement of non-coding DNA elements in pathogenic phenotypes.

Joseph Willson

ORIGINAL ARTICLE Kvon, E. Z. et al. Comprehensive in vivo interrogation reveals phenotypic impact of human enhancer variants. *Cell* https://doi.org/10.1016/j.cell.2020.02.031 (2020)

TECHNOLOGY

Transcriptional profiling of physically interacting cells

Characterizing cellular interactions in vivo and at high resolution remains challenging. Although single-cell RNA sequencing (scRNA-seq) approaches provide high resolution, the required single-cell suspension hampers the study of cell–cell interactions. A new technology, named PIC-seq, combines cell sorting of physically interacting cells (PICs) with scRNA-seq and computational modelling to profile cellular interactions and their impact on gene expression.

PIC-seq applies a mild tissue dissociation protocol that retains some of the in situ cellular structures. Fluorescence sorting by mutually exclusive cell type markers yields a mixture of cell aggregates, representing putative PICs, and single cells of the same cell types. Massively parallel scRNA-seq generates gene expression profiles for PICs that are then computationally deconvolved, by inferring their transcriptional states and contrasting those of non-interacting single cells.

Giladi, Cohen et al. assessed their method by interrogating interactions between T cells and dendritic cells (DCs), using TCRβ and CD11c as markers, respectively. The physical interaction between T cells and DCs represents a prototypical interaction within the immune system and is essential for the distinction of self and non-self antigens. Analyses of cells and their gene expression profiles grown in monoculture, and in co-culture as single cells or cells contributing to PICs, across three time points, showed that T cell–DC interactions were required for T cell activation, proliferation and differentiation.

To determine the in vivo applicability of PIC-seq, the team focused on physical interactions between immune cells and epithelial cells in mouse neonate lungs, which were distinguished using the pan-lineage surface markers CD45 and EPCAM, respectively. Using this model of lung development, the authors were able to assess the crosstalk in epithelial–immune PICs as well as downstream gene regulatory programmes. For example, PICs were enriched for premature alveolar macrophages, monocytes and DCs in neonate lungs, whereas monocyte-derived macrophages, neutrophils and lymphocytes showed little interaction with epithelial cells. Next, the authors looked at T cell–DC interactions and corresponding gene expression profiles in mouse draining lymph nodes during homeostasis and after infection, which highlighted a subset of regulatory T cells involved in these physical interactions, and revealed unique co-stimulatory genes induced in antigen-presenting PICs.

While caveats remain — for example, transcriptional profiles of interacting cells need to be sufficiently 'distant' for deconvolution by the PIC-seq algorithm — this technology shows potential for the in vivo molecular characterization of cell–cell interactions.

Linda Koch

ORIGINAL ARTICLE Giladi, A. et al. Dissecting cellular crosstalk by sequencing physically interacting cells. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-020-0442-2 (2020)