DEVELOPMENTAL BIOLOGY

Tracking development with DNA 'scars'

Alemany, A., *et al. Nature* **556**, 108–112 (2018).

A new technique exploits CRISPR/Cas9 gene editing to trace lineages of mature cells in adult zebrafish. Known as ScarTrace, it relies on 'scarring' of eight tandem green fluorescent protein (GFP) sequences engineered into the fish. After the fish develop into adults, their cells bear different combinations of scars that trace them back to an unknown progenitor cell present early in development.

The method introduces the Cas9 protein, or mRNA that encodes it, into the developing embryo when it is just a single cell, along with a single-guide RNA that directs Cas9 to GFP, where it causes a break in the DNA. The cell repairs the break, leading to an insertion or deletion. This unique "scar" in one of the GFP sites forms when the first embryonic cell divides to two daughter cells. This might happen immediately after delivery if the Cas9 protein is used, but can be delayed a few cell cycles if researchers deliver mRNA instead.

Further Cas9-induced scarring occurs only at cell division, so cells during early embryonic development gradually accumulate scars that their daughter cells inherit. When daughter cells in turn divide, they may accumulate another scar at another GFP.

By the time Cas9 becomes inactive after a few hours, many of the embryo's progenitor cells have a barcode of scars at GFP sites. In a 3-month old adult fish, about 70% of mature cells bear scars. Any adult cell with an identical pattern of GFP scars must be descended from the same progenitor cell that appeared early in development, so the researchers then developed an automated method to sequence the scars. The technique simultaneously categories the adult cells into broad cell types, such as immune cells, based on their messenger RNA expression profile. That let the researchers group adult cells both by cell type and their progenitor, revealing new information about their origins.

In the caudal fin of the adult fish for example, the researchers found a small population of immune cells that had a completely different scar pattern than other blood cells, suggesting that they had come from some other progenitor. To investigate the origin of these cells, the team is conducting experiments in other adult fish organs to see if similar populations exist. If so, "where do they come from? Are they tissue-specific, or do they come from a common origin (and then migrate to tissues)?," asks co-author Anna Alemany, a postdoc at the Hubrecht Institute in The Netherlands.

The technique could potentially be applied to other models, such as mice, but delivering Cas9 at the right developmental stage is still a technical challenge. But at least in the zebrafish, ScarTrace can lend powerful insights into embryonic development. "It's very straightforward and gives you high-throughput data," says Alemany.

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