HHS Public Access

Author manuscript

Science. Author manuscript; available in PMC 2018 August 02.

Published in final edited form as:

Science. 2018 February 02; 359(6375): 521–522. doi:10.1126/science.aar6335.

Tracing single-cell histories:

Origins of mutations in single cells during human brain development and aging are revealed

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DNA mutations accumulate at a steady pace across the human genome, passing from one generation to another. Based on the degree of shared mutations, a genealogical relationship can be reconstructed from ancient and modern individuals, allowing one to go back hundreds of thousands of years in human evolutionary history (1). Instead of comparing individuals, on pages 550 and 555 of this issue, Bae *et al.* (2) and Lodato *et al.* (3), respectively, assessed the rate of DNA mutation in single cells from developing and aging human brains, revealing mutational histories in neurodevelopment, aging, and neurodegeneration. Such approaches also have implications for understanding complex diseases that could result from somatic mutations that arise later in life, such as cancer.

De novo mutations in the DNA of egg or sperm can be associated with devastating disorders affecting young individuals (4). These germline mutations are widely detected as all cells in the body inherit them. By contrast, somatic DNA mutations sporadically occur throughout the life of an organism (that is, post-zygotically) due to DNA damage and errors in DNA replication or repair. When somatic mutations occur early in life in dividing cells, they are found in a large number of cellular descendants. If mutations occur in dividing cells as humans age, they are found in only a limited number of cells, resulting in tissue mutational mosaicism (see the figure). The inheritance pattern of mutations in cells within a tissue can be used to establish a temporal or genealogical relationship of mutations to better understand the role of mutational mosaicism in human diseases. Bae *et al.* analyzed the rate and origin of somatic mutations in the brain prior to birth that could predispose them to functional alterations, including neuropsychiatric or neurodevelopmental disorders. Lodato *et al.* address whether mutations continue to occur later in life and whether DNA mutagenesis in non-dividing cells is associated with neurodegenerative disorders.

To study somatic mutations that arise in development or during aging, an accurate single-cell whole genome sequencing (WGS) method is necessary. This method requires sensitive whole genome amplification techniques (5); however, DNA bases are susceptible to damage (which can lead to mutation), and enzymes used in amplification introduce additional errors. In fact, these false-positive single nucleotide mutations (also referred to as SNVs) can be as high as 10^4 in single-cell WGS (5), vastly outnumbering naturally-occurring SNVs (10^2–10^3/cell). Even in cancer or population genome sequencing projects, mutagenic DNA damage can be a major source of sequencing error for rare variants (6).

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Building on previous methodology (7), Bae *et al.* addressed this challenge by adopting a single-cell cloning method using cultured neuronal precursors from three human fetal brains at 20 weeks of gestation (2). If a cell contains a true heterozygous mutation, the 'wild-type' and mutant allele in the genome should each comprise ~50% of the cell population even after multiple cell divisions. If mutations occur during cell culture or DNA amplification, the frequency of the heterozygous mutation drops to less than 25%. With this criterion, Bae *et al.* sequenced single cells from 31 clonal cultures, identifying somatic mutations by performing clone-to-clone and clone-to-tissue comparisons. This reduced the rate of false positives to 5% and false negatives to 17% in single-cell clones.

Subsequently, they found 200–400 mosaic SNVs in cultures of each neuronal precursor, with the most common substitution being C to A transversion (common in oxidative damage) and 5-methyl-C to T transition (common in germline SNVs). Assuming a linear increase in somatic mutations over time, they estimate 8.6 mutations per cell division, a significant increase compared to mutation rate estimates in early post-zygotic cell divisions (1.3 mutations per cell division) (8, 9). Extending these estimates to regions where neurogenesis continues throughout life (for example, in the hippocampus), Bae *et al.* hypothesize that mutational mosaicism arising from dividing neuronal precursors over time may alter neuronal function in the mature brain for processes such as memory and may thus contribute to neurodegeneration.

Lodato *et al.* took a different approach. Examining post-mitotic neurons, they used an algorithm to extrapolate the frequency of somatic mutation using nearby known germline SNVs. With this method, mutations caused by DNA damage or amplification errors occur on one DNA strand but not the other, whereas naturally occurring somatic mutations are present on both strands (3). Although only mutations near germline SNVs can be discovered this way, it does not require cell culture nor clonal expansion of individual cells, as in other approaches (2, 7).

They then carried out single-cell WGS on 93 normal post-mitotic prefrontal cortex (PFC) neurons in individuals from 4 months to 82 years of age, 26 normal hippocampal dentate gyrus neurons, and 42 PFC neurons from patients with DNA damage repair-deficient neurodegenerative disorders. Strikingly, they found 300–900 SNVs per cell in PFC neurons within one year of birth, consistent with the estimate provided by Bae *et al.* In addition, they observed a two-fold higher rate of somatic mutations in hippocampal dentate gyrus neurons (with 40 mutations occurring per cell each year) than in PFC neurons, as was also proposed by Bae *et al.* Furthermore, patients with DNA damage repair-deficient neurodegenerative disorders showed a two-fold increase in somatic mutations in post-mitotic neurons across different age groups. They also detected three distinct mutational signatures in all of the cells studied, suggesting multiple mechanisms of somatic mutation associated with brain development, aging, and neurodegeneration.

Although these studies do not provide definite mechanisms of how somatic mutations occur or establish their functional consequences, it is exciting that Bae *et al.* and Lodato *et al.* utilized readily accessible methods to accurately sequence single-cell whole genomes and reconstructed mutation histories (akin to a 'mutational clock') with single-cell resolution.

Because recent mutations are restricted to the tiniest mosaic pieces (see the figure), mutational clocks had previously been limited to the early mutation events shared by many cells. These technological advances in single-cell WGS allow for examining recent, uncommon mutational events unique to each cell, considerably widening the temporal breadth of mutation history reconstruction.

In so doing, fundamental biological questions that had been inaccessible can now be addressed, especially regarding the onset and progression of relatively recent somatic mutations in development or in aging. In fact, the studies here have now firmly established the presence of ongoing DNA damage later in life that also accelerates in neurodegeneration (3). They also demonstrate the occurrence of coding and non-coding mutations that arise later during neurogenesis (2), potentially dividing the brain into smaller mutational territories with implications for the brain connectivity, function, and predisposition to neurological disorders.

The accuracy of single-cell WGS continues to improve, including new amplification methods with a lower error rate and more uniform coverage of the genome (5). Unfortunately, single-cell WGS is limited to a small number of cells due to throughput and cost bottlenecks. In order to accurately reconstruct mutation history and mosaicism throughout life, it is necessary to trace cell lineages comprehensively. Recently, somatic alterations induced by members of the CRISPR-associated (Cas) family of DNA nucleases have been used to reconstruct cell lineages in model organisms or cell lines (10, 11). In this method, Cas9-induced alterations within a single synthetic locus can be traced. Such reporters are transcriptionally active, making them compatible with high-throughput single-cell RNA sequencing (12) to ‘read’ the reporter and identify cell type-specific RNA signatures. In fact, reporters can even be programmed to record sequential events in cells (13), potentially revealing environmental interactions that shape the tissue hierarchy and cell lineages within.

One day, it might be possible to engineer cell lineage and activity recorders throughout an entire organism, followed by comprehensive single-cell sequencing of recorders containing induced mutations. It might then be possible to compare naturally-occurring somatic mutations in a subset of single cells using WGS across specific developmental stages. This could reveal biological processes that affect the mutational clock across different tissue types or cellular activities and expand our understanding of mutational signatures (14, 15), especially if such signatures are conserved across species. Precisely answering when, how, and where mutational events occur in cells within different tissues will help clarify important questions in development, aging, and disease research.

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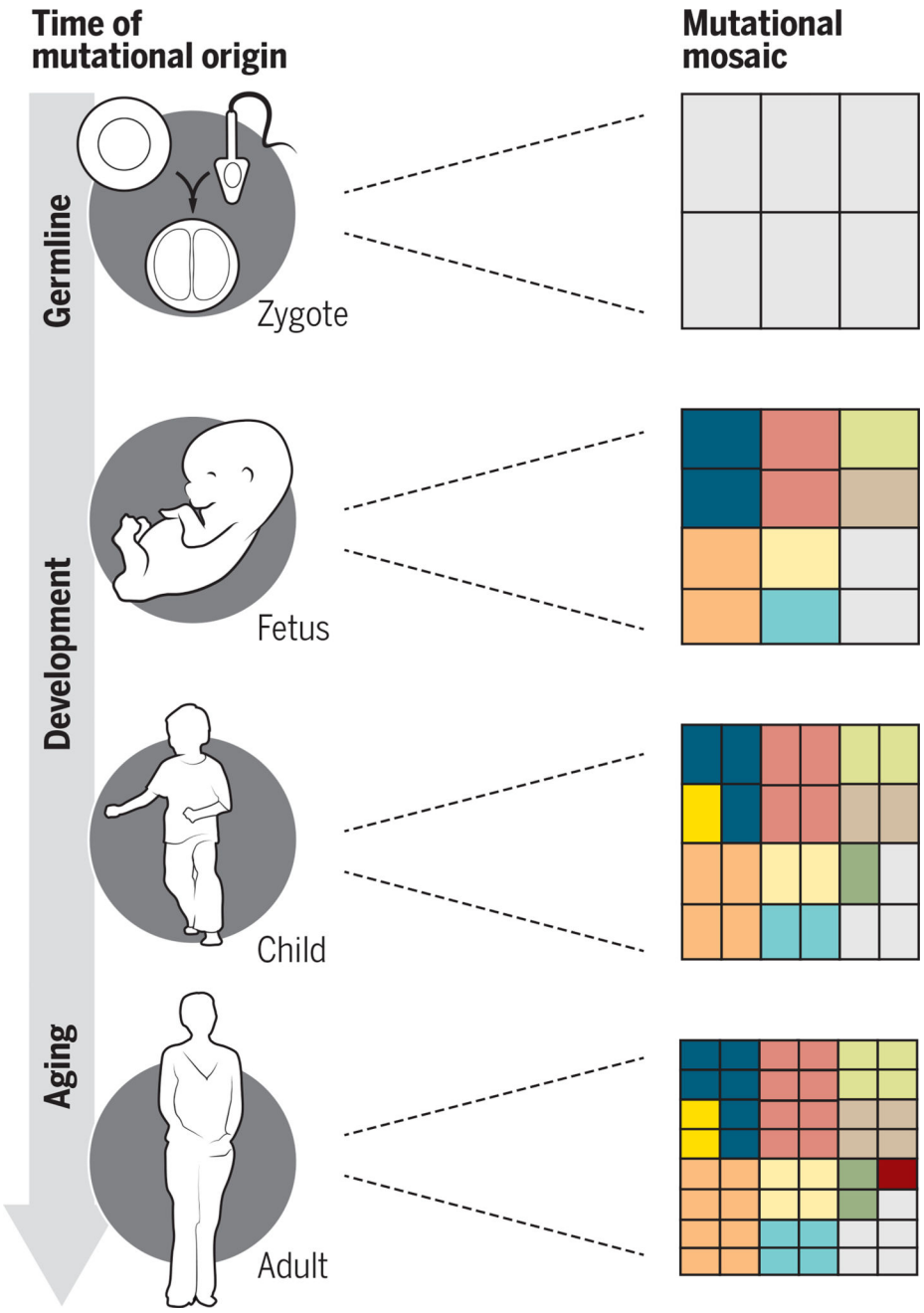

figure 1. Reconstructing cellular mutation history

Unique mutations can be present in most or just in one cell within a tissue. Emerging sequencing technologies can accurately profile genome wide somatic mutations even in the tiniest puzzle piece, a single cell.