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Review

# Functional analysis of cell plasticity using single-cell technologies

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**Metazoan organisms are heterocellular systems composed of hundreds of different cell types, which arise from an isogenic genome through differentiation. Cellular 'plasticity' further enables cells to alter their fate in response to exogenous cues and is involved in a variety of processes, such as wound healing, infection, and cancer. Recent advances in cellular model systems, high-dimensional single-cell technologies, and lineage tracing have sparked a renaissance in plasticity research. Here, we discuss the definition of cell plasticity, evaluate state-of-the-art model systems and techniques to study cell-fate dynamics, and explore the application of single-cell technologies to obtain functional insights into cell plasticity in healthy and diseased tissues. The integration of advanced biomimetic model systems, single-cell technologies, and high-throughput perturbation studies is enabling a new era of research into non-genetic plasticity in metazoan systems.**

## Highlights

Cell plasticity describes the ability of cells to alter their phenotypes in response to external stimuli without changing their genotypes.

Cell plasticity is essential for metazoan tissue biology and has significant clinical implications.

The study of cell plasticity requires experimental models that are amenable to systematic functional perturbations.

Single-cell technologies enable the study of cell plasticity across multiple modalities, allowing the construction of functional plasticity phenoscapes.

## Terminology and concepts

Metazoan organisms comprise multiple **cell types** (see Glossary) that each perform discrete functions. To achieve such intricate **heterocellularity** from an isogenic genome, pluripotent stem cells must differentiate into distinct cell types. Cell type transitions can also occur outside of the canonical **differentiation** process, through a mechanism loosely termed '**plasticity**'. Cell plasticity refers to the ability of cells to modify their phenotypes in response to environmental signals without altering their genotypes [1]. Given the importance of cell fate dynamics in wound healing, infection, and cancer, there is substantial interest in understanding the mechanisms that govern cell plasticity. This review will explore state-of-the-art model systems and analytical technologies to study cell plasticity mechanisms. However, before discussing methods to study plasticity, it is essential to make three important clarifications.

First, the phenotypic change driven by cell plasticity should present itself as alterations in cell types. A cell type represents a relatively stable functional unit within an organism, maintaining tissue architectures while performing specific biological functions [2]. A closely linked, yet distinct facet of cellular phenotypes is the **cell state**. From our perspective, the cell state embodies a more dynamic and occasionally transient phenotypic space that does not require a change in cell type. For example, a cell can exhibit various cell states, such as proliferative, quiescent, or dying, regardless of its cell type or as it goes through cell type transitions. Similarly, we do not consider immune cell plasticity or neural plasticity in this review as they are more pertinent to changes in cell states than cell types.

Second, cell plasticity describes the ability of a cell to change its cell type in response to external stimuli. This distinguishes cell plasticity from cell potency, which is an inherent characteristic of stem or progenitor cells observed during development and tissue regeneration [3]. In this sense, cell plasticity is restricted under homeostasis and only becomes activated when the tissue steady state is perturbed or disrupted by cell-extrinsic cues.

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Last, cell plasticity is a quantifiable trait that represents the ease with which cells can deviate from their steady-state identity and adopt alternative fates (Figure 1A). A cell with high plasticity can easily change cell type in response to cell-extrinsic cues, whereas a cell with low plasticity cannot. Pioneering efforts have been made to estimate cell plasticity using single-cell transcriptomics [4] and **lineage tracing** [5], while ongoing advances in multiomics techniques [6,7] hold the potential to standarise the quantification of cell plasticity.

## Types of cell plasticity

Although cell phylogeny is typically considered a top-down structure driven by tissue-specific differentiation, it is important to note that this hierarchy can be traversed or even reversed under certain circumstances, such as injury or disease [8]. Depending on the nature and direction of cell type transitions, cell plasticity can manifest in three forms: **transdetermination**, **transdifferentiation**, and **dedifferentiation** [9] (Figure 1B).

Transdetermination is the process by which distinct but related populations of stem or progenitor cells convert into one another [10]. This phenomenon has been most detailedly described in *Drosophila*, where leg-to-wing conversions occurred reproducibly after transplantation of the imaginal disc. Similar transdetermination events can be induced *in situ* by ectopic expression of the *wingless* gene, suggesting that transdetermination is a cell-inherent potential that can also be activated cell-extrinsically [11]. Transdetermination has not been extensively studied in vertebrates.

Transdifferentiation refers to the interconversion between differentiated cell types, with or without the involvement of an intermediate progenitor-like stage [8]. Classic examples of transdifferentiation include **epithelial-mesenchymal transition(EMT)** manifested in tissue fibrosis (type 2 EMT) or cancer invasiveness (type 3 EMT) [12].

Transdetermination and transdifferentiation represent 'horizontal' cell type transitions. By contrast, dedifferentiation describes a 'vertical' cell type transition where differentiated cells revert to a progenitor-like state [8]. A classic example of dedifferentiation is the generation of **induced pluripotent stem cells (iPSCs)** from terminally differentiated cells through epigenetic reprogramming [13]. Dedifferentiation can also occur under pathological conditions, such as tissue damage, where newly generated progenitor cells divide, differentiate, and repopulate the damaged tissue, as has been observed in the intestine [14], pancreas [15], and liver [16].

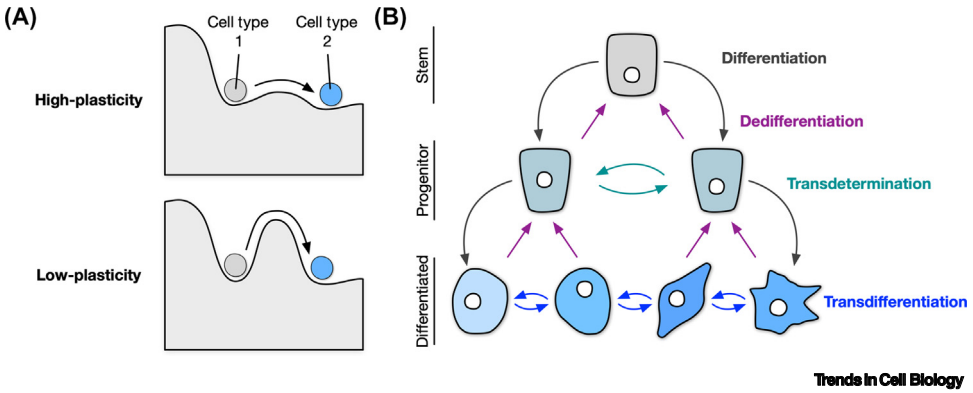


Figure 1. Terminology and concepts. (A) Cell plasticity describes the ease with which cells transition between different cell types. The barrier for cell type transition is relatively low for high-plasticity cells and, conversely, relatively high for low-plasticity cells. (B) Definitions of differentiation, dedifferentiation, transdetermination, and transdifferentiation. While differentiation is driven by cell potency, the others are underpinned by cell plasticity.

## Glossary

**ATAC-seq:** (assay for transposase-accessible chromatin using sequencing), a method to assess genome-wide chromatin accessibility by inserting sequencing adapters into open chromatin with the hyperactive mutant Tn5 transposase.
**Atlas study:** a static method to characterise the composition and distribution of all cells of a tissue, organ, or organism with spatial and/or temporal resolution.
**Cell state:** a condition or status of a cell type (e.g., cell-cycle stage).
**Cell type:** a specific form of a cell defined by its development, structure, gene expression, and function.
**Dedifferentiation:** a process in which differentiated cells lose their specific characteristics and revert into a more stem cell-like state.
**Differentiation:** the process by which cells or tissues change from a progenitor state to specialised types through the regulation of gene expression, often during development.
**Epigenomics:** the study of chemical modifications to the DNA and histone proteins of an organism without altering the underlying DNA sequence.
**Epithelial-mesenchymal transition (EMT):** a process in which epithelial cells obtain mesenchymal characteristics such as mobility in wound healing and invasiveness in cancer.
**Fate mapping:** a method originating from developmental biology to understand the origins of different cell types in an adult organism, typically applied at the tissue level.
**Functional study:** an experimental approach that focuses on investigating the dynamic functions of the biological system of interest, rather than its static profiles.
**Heterocellularity:** a population of cells comprising different cell types.
**Induced pluripotent stem cell (iPSC):** a type of pluripotent stem cell that can be generated through epigenetic reprogramming of differentiated adult cells.
**Intratumour heterogeneity:** the genetic and phenotypic variation observed among different cells within a single tumour, which can influence tumour growth, progression, metastasis, and response to treatments.
**Lineage tracing:** a technique for reconstructing cell phylogenies by tracing cell division, differentiation, and

## Cell plasticity model systems

The canonical definition of a cell type entails a static representation of the cellular phenotype and its developmental history [2]. However, an emerging perspective on cell type also considers a cell's response to external perturbations integral to its identity [17]. To mechanistically study cell plasticity, a model system should: (i) recapitulate the entire sequence of events involved in plasticity-driven cell type transitions; (ii) allow noninvasive but accurate monitoring of the transition, preferably in a spatiotemporally resolvable manner; and (iii) be amenable to systematic perturbations and subsequent **functional studies** (Figure 2). Static analysis of human biopsies can identify cell types and states [18] but cannot elucidate the functional mechanisms driving cell type transitions and, thus, is not considered a plasticity model in this review. In this section, we will discuss the study of cell plasticity *in vivo* using classic model organisms and mouse models of tissue damage and diseases, as well as *in vitro* using immortalised cell lines, iPSCs, and **organoids.**

### Classical model organisms

Early observations of cell plasticity can be traced back to the 1700s, with notable examples such as the ability of freshwater hydra to regenerate following bisection and the regrowth of amputated limbs by salamanders [9]. Research on simple organisms continues to be vital in advancing our understanding of cell plasticity, thanks to their amenability to culture and manipulation, as well as our in-depth knowledge of their genetics, anatomy, and development. For example, *Caenorhabditis elegans* was initially recognised as a model for developmental biology, but has also contributed to our understanding of wound healing, tissue regeneration, and cell plasticity associated with transdifferentiation [19]. The *Drosophila* imaginal disc has been utilised to investigate transdifferentiation and dedifferentiation, while the transparent body and rapid development of zebrafish have made them excellent models to functionally analyse cell state transitions in melanoma [20].

### Mammalian models

Classical model organisms have played a crucial role in uncovering the molecular mechanisms of metazoan cell plasticity, but the direct applicability of these models to human biology can be obscured by their inherent simplicity. To investigate cell plasticity in a context more closely aligned with human biology, a variety of mammalian models, particularly mouse models, have been developed.

Tissue regeneration is a complex biological process that involves the active deployment of cell plasticity. When tissue damage is induced in a lineage-traceable manner, it is possible to track cell lineages and phenotypes as the tissue homeostasis is being restored. Tissue damage can be induced by a variety of methods, including cell type-specific expression of the diphtheria toxin receptor [15], high-dose irradiation [21], dietary administration of toxic substances [22], and tissue-specific diseases [23]; all can be used as cues for plasticity studies.

In addition to cell plasticity in tissue regeneration, mouse models are also widely used to study cell plasticity in cancer [5,24,25]. Fumagalli *et al.* developed a model of human colorectal cancer (CRC) xenografts to investigate the plasticity of CRC cells during metastasis, demonstrating that CRC spreads and recolonises as $LGR5^-$ cells while regaining their $LGR5^+$ cancer stem cell identity during metastatic outgrowth [25]. In another study, Yang *et al.* used a genetically modified mouse model (GEMM) of *Kras/Trp53*-driven lung adenocarcinoma (LUAD) to trace the progression of cancer from a single transformed cell to metastatic tumours, highlighting the contribution of cell plasticity in dictating the evolutionary trajectories of LUAD [5]. Intriguingly, the authors demonstrated that the frequency of changes in cancer cell fates, effectively their plasticity, can be estimated from tumour phylogenies. This dynamics-driven approach distinguishes lineage plasticity

migration with the help of incorporated cell lineage labels.
**Modality:** refers to cellular molecules such as DNA, RNA, and proteins, in the context of single-cell omic technologies.
**Omics:** collective description of large numbers of cellular molecules such as genes, proteins, and RNAs.
**Organoid:** self-organising stem cell-derived *ex vivo* cultures widely adopted as biomimetic models of healthy and diseased tissues.
**Persister cancer cells:** cancer cells characterised by their slow proliferation, flexible energy consumption, adaptation to the microenvironment, phenotypic plasticity, and resistance to treatment.
**Phenoscape:** the totality of all possible phenotypes a cell can assume, both under steady state and following perturbations.
**Plasticity:** the ability of cells to transition into alternative cell types in response to environmental cues without changing their genotypes.
**Plasticity landscape:** a conceptual framework to illustrate the range of states a cell can acquire and its potential to transition into different cell types, determined by genetic, epigenetic, and environmental factors.
**RNA velocity:** a high-dimensional vector that predicts future states of single cells based on profiles of unspliced and spliced mRNA.
**Signalling entropy:** a single-cell metric of gene expression relative to predicted protein–protein interaction networks. Pluripotent cells have high signalling entropy, whereas differentiated cells have low signalling entropy.
**Single-cell RNA-sequencing (scRNA-seq):** a molecular biology technique to examine the transcriptome from individual cells.
**Split-and-pool:** a combinatorial process that comprises the mixing and separation of intermediate products, leading to an exponential increase in the number of products.
**Transdetermination:** a process in which distinct but related populations of stem or progenitor cells transition into one another.
**Transdifferentiation:** a process in which a differentiated cell transitions into a different cell type, with or without entering an intermediate progenitor-like state.

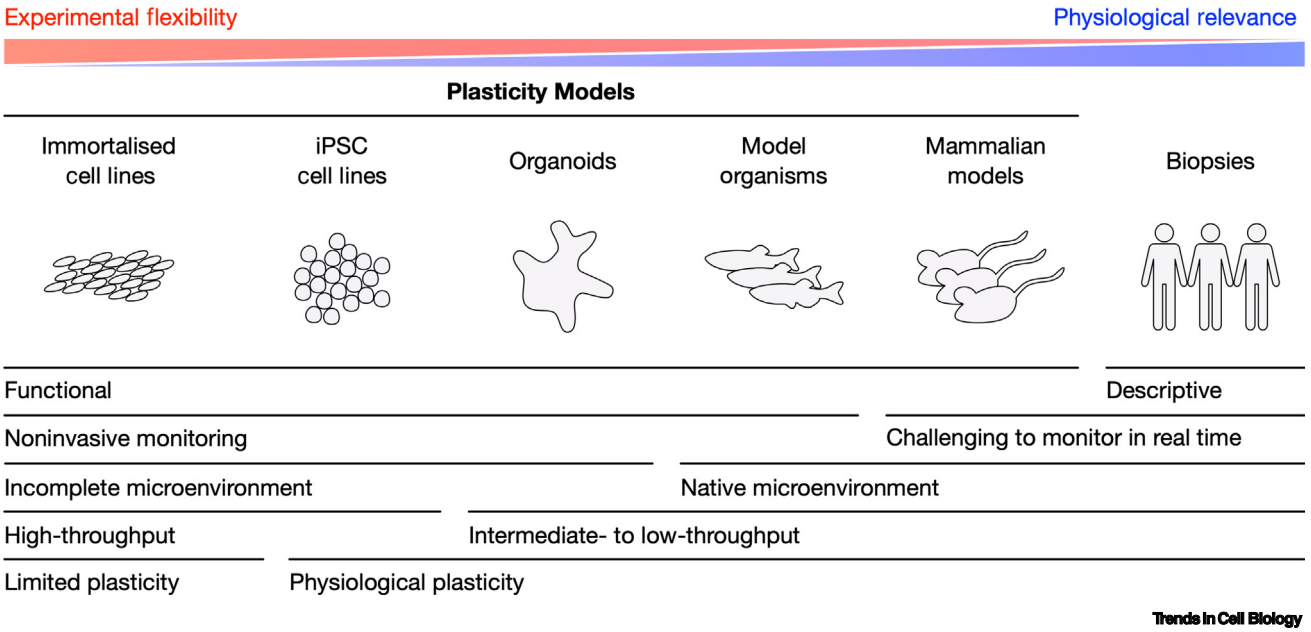


Figure 2. Cell plasticity model systems. In contrast to static human biopsies that only allow descriptive analysis, plasticity models are amenable to experimental perturbations, enabling systematic functional studies. *In vitro* cultures, such as 2D cell lines and 3D organoids, allow real-time monitoring and continuous interrogation of plasticity-driven cell type transitions, yet they do not usually represent the native microenvironment. Notably, organoids exhibit physiological plasticity reminiscent of their tissue of origin and are compatible with intermediate-throughput analysis, making them a powerful model system for plasticity studies. Abbreviation: iPSC, induced pluripotent stem cell.

from **intratumour heterogeneity**, providing an essential tool to understand the roles of cell plasticity in subclonal expansion during tumour progression.

### *In vitro* models

Beyond simple organisms and mouse models, cell plasticity can also be studied using *in vitro* experimental systems such as immortalised cell lines, iPSCs, and organoids. The simplicity of these models is ideal for high-throughput investigation of cell plasticity, particularly for biological processes that are challenging to systematically interrogate *in vivo* [26]. In an attempt to assess the suitability of cancer cell lines to model tumour heterogeneity, Kinker *et al.* conducted a comprehensive single-cell transcriptomic analysis of approximately 200 cancer cell lines across 22 cancer types, revealing 12 recurrent gene expression programmes reminiscent of human tumours, including one programme associated with EMT [27]. Moreover, the generation and optimisation of iPSC lines have laid a solid foundation for regenerative medicine and cell therapies by revealing plasticity mechanisms of dedifferentiation and differentiation [13].

Despite their tractability, it is important to note that 2D cell lines have limited physiological relevance. By contrast, organoids are 3D self-organising systems that retain the differentiation and plasticity of their tissue of origin, as has been demonstrated with the lung [28], pancreas [29,30], and bladder [31]. Furthermore, organoids strike an excellent balance between biological fidelity and experimental flexibility, making them especially suitable for systematic mechanistic studies [26]. For example, we recently reported a highly multiplexed single-cell drug screening of CRC patient-derived organoids in monoculture or co-cultured with cancer-associated fibroblasts (CAFs). Through single-cell profiling of ~2500 organoid cultures, we found that CAFs can exploit CRC cell plasticity and polarise cancer cells towards a slow-cycling revival stem cell

fate, protecting them from chemotherapy [32]. Collectively, these studies underscore the potential of organoids as a versatile model for cell plasticity research.

## Methods to study cell plasticity

Model systems are only as powerful as the methods used to study them. Cell plasticity is a dynamic process and is best studied using process-orientated approaches, such as phenotypic monitoring (to describe plasticity outcomes) and lineage tracking (to record plasticity in action). Consequently, static cell **atlas studies** are often inadequate to generate a comprehensive understanding of cell plasticity; a more dynamic and functional approach is needed (Figure 3).

### Phenotyping

When applied to cell plasticity models, continuous phenotypic monitoring can be achieved using techniques such as microscopic and macroscopic observation, as well as molecular characterisation. For example, Ireland *et al.* used time-series **single-cell RNA sequencing (scRNA-seq)** to explore the development of intratumour heterogeneity during human small cell lung cancer tumourigenesis, demonstrating that MYC signalling can drive a dynamic tumour evolution programme [33]. Using high-dimensional single-cell profiling of more than 1000 organoid monocultures and co-cultures, we recently charted a continuous perturbation **phenoscape** of colonic stem cell polarisation driven by cell-intrinsic and -extrinsic factors, demonstrating that oncogenic signalling restricts cancer cell plasticity in the tumour microenvironment (TME) and traps CRC cells in a cancerous state [34]. By integrating dimensionality reduction [35], **RNA velocity** [36], and **signalling entropy** [37], it was possible to visualise global cell potency and local cell plasticity on a Waddington-like landscape [34].

Although phenotypic characterisation can serve as a proxy for cell plasticity, it does not delineate how one cell type transforms into another, and the detection of intermediate cell states largely relies

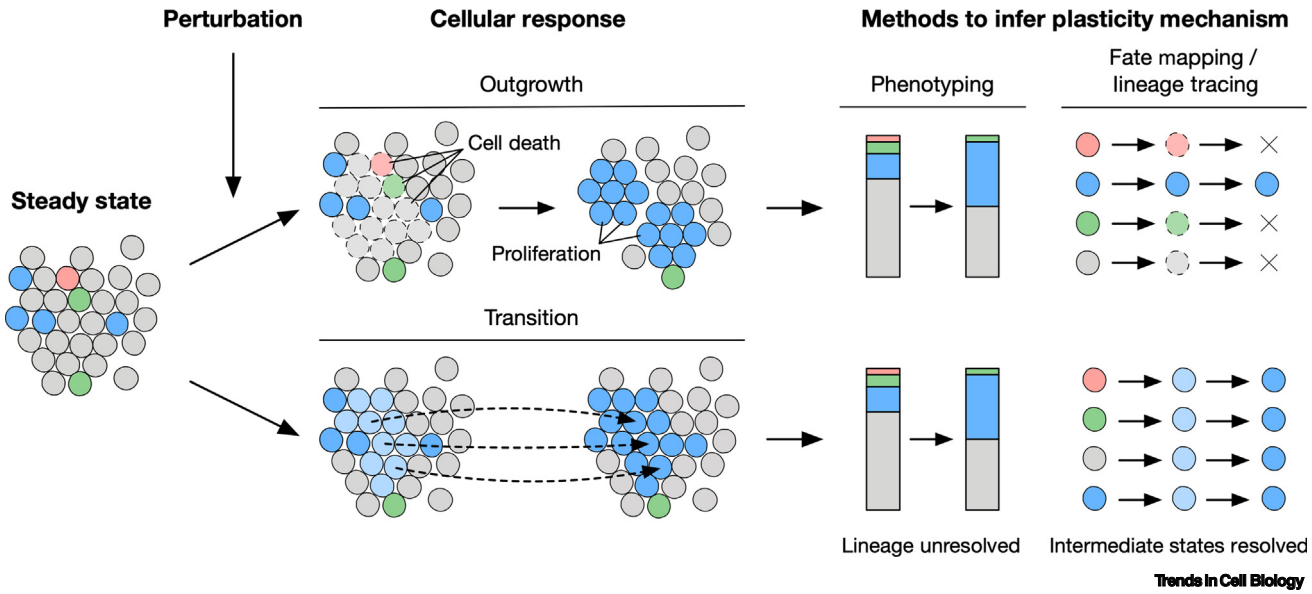


Figure 3. Methods to study cell plasticity. While the tissue steady state can be described by traditional static methods, cell plasticity can only be revealed with perturbation, necessitating a dynamic and functional approach. Phenotyping can capture changes in cell type composition but not cell lineage information, thus preventing the distinction between different population dynamics, such as cell outgrowth driven by competition and cell type transition driven by cell plasticity. By contrast, fate mapping and lineage tracing allow the inference of intermediate cell states, enabling the differentiation between different modes of perturbation-induced alterations in cellular dynamics.

on the timing of the observations (Figure 3). An alternative approach is to incorporate inheritable reporters that automatically record the lineage history as cells undergo phenotypic changes. Two widely adopted methods in this category are **fate mapping** and lineage tracing [38].

### Fate mapping

Fate mapping originated as a method in developmental biology to understand the embryonic origins of adult tissues and structures [39]. In a pioneering study conducted in 1905, E.G. Conklin injected dyes into four-cell stage ascidian embryos, tracked their migration, and generated the first-ever fate map of a multicellular organism, offering valuable insights into the predetermined nature of development [39]. Later advances in genetic labelling led to more sophisticated fate mapping techniques. For example, Snippert *et al.* developed the Cre-loxP multicolour Confetti mouse model and collected clonal tracing data of individual LGR5$^{hi}$ intestinal stem cells, unveiling a neutral drift model of crypt clonality [40]. Since then, the repertoire of multicolour mouse models has expanded significantly, allowing high-resolution fate mapping in increasingly sophisticated biological settings [41].

### Lineage tracing

While fate mapping is valuable for tracking cells along their developmental trajectories, it is primarily used at the tissue level and does not allow lineage inference of single cells. To precisely reconstruct the progeny trees (i.e., the complete history of cell division, differentiation, and migration) originating from individual cells, a variety of lineage tracing techniques have been developed [38]. As with fate mapping, cell lineages can be traced through visual observation, but this approach is only feasible for simple model systems [39]. For more complex biological settings, naturally heritable genetic markers provide a robust and reliable method for lineage tracing. For example, McKenna *et al.* took advantage of the CRISPR/Cas9 system and conceived an innovative technique to record cell lineages during the development of zebrafish. The key feature of this method is the iterative editing of a synthetic construct over time, resulting in the accumulation of distinct genetic 'scars' that can be used to reconstruct cell phylogenies through targeted DNA and RNA sequencing [42]. This method has been successfully adapted for single-cell applications [43,44], opening up new avenues to understanding how cell plasticity influences cell fate determination and population dynamics.

## Functional single-cell approaches for cell plasticity research

In recent years, remarkable progress has been made in the field of single-cell technologies, covering a wide range of cellular **modalities** such as the genome, transcriptome, proteome, DNA and chromatin modification, chromatin accessibility, and CRISPR perturbation arrays [45]. Unlike traditional bulk analysis that homogenises complexity, single-cell technologies preserve heterocellularity, a crucial prerequisite for identifying the nuanced cell states driven by cell plasticity. Recent advances in genetic barcoding further facilitate the tracking of cell lineages at single-cell resolution [46], while the simultaneous analysis of multiple cellular modalities has enabled the deduction of gene regulatory networks [47]. In this section, we will discuss the current state and future directions of cell plasticity research, with a particular focus on the integration of single-cell technologies and functional interrogations.

### Single-cell transcriptomics

Of all single-cell **omics** technologies, scRNA-seq is the most mature and widely utilised method for high-dimensional profiling of cellular identities across diverse tissue types. The ability of scRNA-seq to adequately resolve distinct cell types and model cell fate transitions has made it an indispensable tool for understanding cell plasticity, as evidenced by its application in a variety of contexts, including wound healing [48], pan-cancer EMT [49], and various cancers such as

melanoma [50], small cell lung cancer [33], LUAD [51], and glioblastoma [52]. For example, to understand the development and maintenance of intratumour heterogeneity, Marjanovic *et al.* performed longitudinal scRNA-seq analyses of lung cancer developed in a series of GEMMs [51]. This study unveiled a high-plasticity cell state (HPCS) characterised by mixed transcriptional programmes, high probabilities of transitioning into alternative LUAD cell fates, and increased resistance to chemotherapy. Importantly, despite being highly plastic, the hyper-proliferative potential of HPCS distinguishes them from slow-cycling '**persister cancer cells**' that underpin tumour heterogeneity in other types of cancer [53]. A comprehensive understanding of how cell plasticity underlies tumourigenic potential and drug resistance is essential for targeting these cells effectively for cancer therapies.

In addition to capturing cell type-specific transcriptomic profiles, scRNA-seq can also be used to elucidate the regulation of cell plasticity through cell–cell communication, which can be putatively inferred from ligand–receptor expression pairing [54]. Analysis of the glioblastoma TME through ligand–receptor interactions demonstrated that macrophages can activate STAT3 signalling in cancer cells and induce a mesenchymal-like (MES-like) cell state. The MES-like glioblastoma cells can reciprocally regulate macrophages and cytotoxic T cells within the TME, suggesting that cancer cell plasticity may have implications for immunotherapies [55]. In another example, single-cell analysis of an autochthonous pancreatic cancer model revealed that epigenetic plasticity of $Kras^{G12D/+}$ cells can be induced by immune–epithelial signalling, which in turn endows more extensive cell–cell communication, ultimately leading to the formation of neoplasia [56]. These studies suggest that the inflammatory TME may be a driving force for cancer cell plasticity and can be leveraged for cancer interventions.

### Single-cell lineage tracing (scLT)

Single-cell technologies have also significantly advanced the field of lineage tracing, offering an integrative framework that combines the labelling of cell fates [43] with the inference of transcriptional dynamics [57,58]. Effective scLT entails three key technical components: (i) the incorporation of unique, inheritable, and retrievable barcodes into specific cell populations; (ii) the identification of cellular barcodes using single-cell technologies; and (iii) the reconstruction of cellular phylogenies through computational analysis [59]. Step (i) may not always be necessary as naturally occurring somatic mutations can serve as inherent cellular barcodes [60,61]. Steps (ii) and (iii) have been greatly facilitated by the introduction of novel barcoding systems such as Watermelon [62], ClonMapper [63], and DARLN [64]. These models not only provide intrinsic lineage tracing capacity through genetic barcoding, which directly validates lineage dynamics inferred from the other modalities, but also enable phenotypic characterisation to generate functional insights into cell plasticity.

scLT has been widely adopted to study cell lineage relationships during development, such as in *C. elegans* [65], zebrafish [66,67], and mice [64,68]. Furthermore, the application of scLT has also empowered functional interrogation of cell plasticity in tissue regeneration [69] and cancer evolution [5,70]. Importantly, scLT has been successfully applied to human iPSC-derived cerebral organoids, whereby He *et al.* leveraged the CRISPR/Cas9-induced scarring system [43] to study human brain development with high temporal-spatial resolution [44]. Given that organoids can serve as an experimentally amenable model for tissue biology, we anticipate that a wider implementation of scLT in organoid studies will yield valuable functional insights into cell plasticity under both healthy and diseased conditions.

### Single-cell epigenomics

While scRNA-seq is a powerful tool for identifying cell types and inferring cell lineages, it only provides an indirect measure of cell plasticity. For example, transcriptomics can describe cell type

transitions in response to external perturbation, but it cannot fully explain the regulatory mechanisms that sculpt the underlying **plasticity landscape** (Figure 4). To better understand the regulation of cell plasticity, it is crucial to incorporate additional omics modalities, such as DNA methylation, histone modification, and noncoding RNAs [71]. This highlights the necessity of integrating single-cell **epigenomics** into cell plasticity studies [72].

sc**ATAC-seq** (single-cell assay for transposase-accessible chromatin using sequencing) is an efficient method to profile chromatin accessibility at single-cell resolution and has become a common first step in epigenomic studies [73]. Recent advances in multiomics technologies have made it possible to analyse gene expression and chromatin accessibility in the same cells, enabling the analysis of cell-type transitions via linked chromatin landscape and transcriptional profiles [6,74]. For example, Ma *et al.* implemented the **split-and-pool** barcoding strategy in the standard scATAC-seq workflow and developed SHARE-seq, a highly scalable approach for simultaneously profiling open chromatin and transcriptomics at single-cell resolution. Using this method, the authors demonstrated that chromatin accessibility primes cell-fate commitment, a mechanistic insight immediately relevant to the regulation of cell plasticity [6]. To our knowledge, the direct application of multiomics single-cell technologies to plasticity models is currently lacking, which presents a unique opportunity to obtain further insights into the regulatory networks governing cell plasticity.

In addition to chromatin accessibility, additional layers of epigenetic regulation such as transcription factor binding, DNA modification, histone modification, and genome topology also play critical roles in determining gene expression and, ultimately, cell plasticity [72]. Despite the challenges posed by multiomics single-cell technologies such as the need for extensive benchmarking, high sequencing costs, and limited coverage of paired single-cell modalities, the ever-evolving landscape of technological and computational advancements is leading towards an integrated functional understanding of cell plasticity.

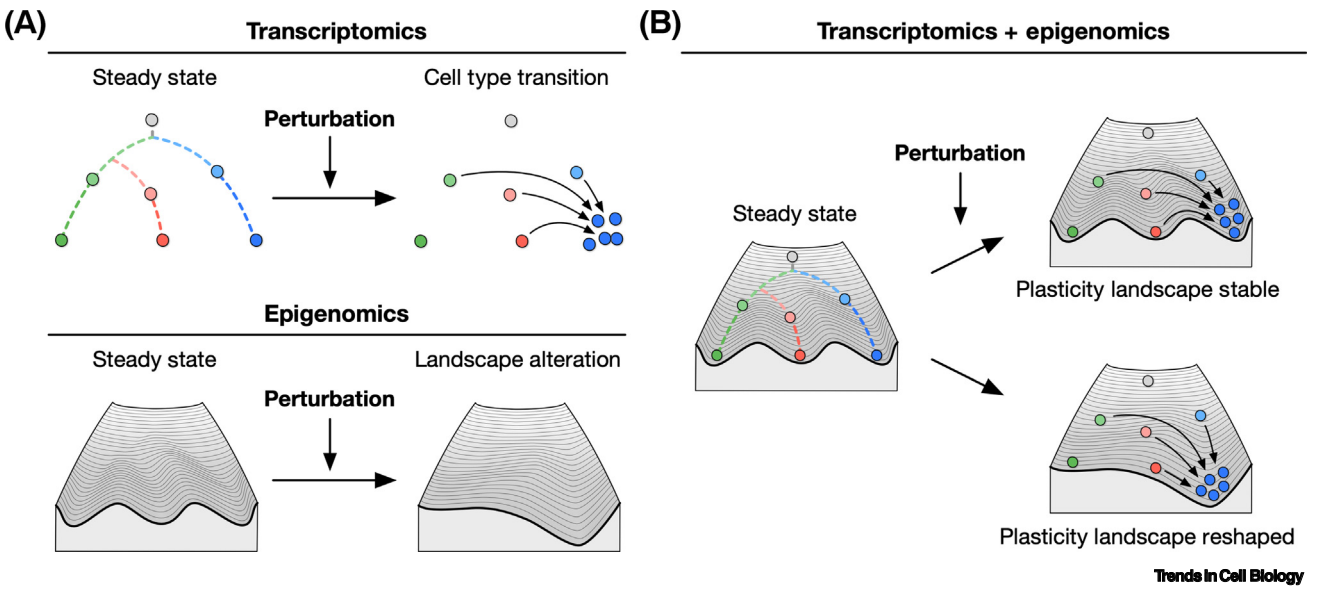


Figure 4. Transcriptomic and epigenomic analysis of cell plasticity. (A) When the tissue steady state is perturbed by external stimuli, transcriptomics describes cell type transitions, while epigenomics uncovers the regulatory mechanisms underlying the topology of the plasticity landscape. (B) Integrated transcriptomic and epigenomic analysis enables a comprehensive understanding of population dynamics driven by cell plasticity, with the plasticity landscape either remaining stable or being modified by the perturbation.

## Concluding remarks

Cell plasticity is a fundamental feature of multicellular organisms with profound translational implications. Just as nucleotide sequencing has revolutionised our understanding of the genetic basis of human diseases, we are now entering an era of non-genetic cell plasticity, thanks to the development of novel experimental models and single-cell technologies (see Outstanding questions).

To obtain a comprehensive understanding of cell plasticity under various conditions, it is crucial to utilise experimental systems that are tractable to systematic interrogation. In this regard, organoids are a powerful platform for plasticity studies given their physiological relevance [75], amenability to experimental manipulation [76], compatibility with sample multiplexing [77], and applicability to lineage tracing [44]. We anticipate that systematic perturbation and functional interrogation of organoid models of both healthy and diseased tissues will offer substantial mechanistic insight into cell plasticity.

Single-cell technologies have been widely used to generate comprehensive molecular maps of complex tissues [45]. In light of the recently proposed definition of cell type encompassing cellular responses to perturbations [17], next-generation tissue atlases should also incorporate cell plasticity inferred from functional analysis (e.g., perturbation studies of their matched organoid models). This orthogonal approach may bridge static biopsies with dynamic experimentation, illuminating the critical modalities required for measuring cell plasticity. Furthermore, the combination of experimental perturbation and multiomics analysis will not only broaden the current specification of cellular phenoscapes [17], but also enable the inference of the gene regulatory mechanisms underlying cell plasticity [71]. Last, advances in spatial transcriptomics and single-cell barcoding techniques represent an exciting avenue for understanding cell plasticity in the context of native tissue [78]. When combined with novel GEMMs [79] and syngeneic models [80], the integrated information on cell lineage, cell location, and molecular characteristics will uncover the temporal-spatial connection between cellular history and population dynamics, allowing the construction of extensive regulatory networks underpinning cell plasticity.

Functional single-cell technologies are designed to capture the intricate heterogeneity of complex biological systems and to decipher the regulatory mechanisms that drive this heterogeneity. We anticipate that the deployment of these technologies to biomimetic experimental systems will revolutionise our understanding of cell plasticity.

## Outstanding questions

Is it possible to monitor cell plasticity in real time?

Can we infer cell plasticity from static biopsies?

How can we incorporate functional interrogation into atlas studies?

What data modalities are necessary to accurately measure plasticity?

Can we generate a universal metric of plasticity?

Can dysregulated plasticity be targeted therapeutically?

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### Declaration of interests

The authors declare no competing interests.

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