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Review

Quantitative Single-Cell Approaches to Stem Cell Research

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Understanding the molecular control of cell fates is central to stem cell research. Such insight requires quantification of molecular and cellular behavior at the single-cell level. Recent advances now permit high-throughput molecular readouts from single cells as well as continuous, noninvasive observation of cell behavior over time. Here, we review current state-of-the-art approaches used to query stem cell fate at the single-cell level, including advances in lineage tracing, time-lapse imaging, and molecular profiling. We also offer our perspective on the advantages and drawbacks of available approaches, key technical limitations, considerations for data interpretation, and future innovation.

Introduction

A fundamental requirement for all stem cell (SC) studies is the clear identification of a cell as a bona fide SC. To this end, a cell's self-renewal and differentiation potential needs to be assessed at single-cell resolution. Single-cell approaches with defined endpoints to read out clonal cell fates have traditionally been an important method in developmental and SC biology (Becker et al., 1963). Because explicit markers that allow the prospective isolation of SCs with highest purity are not yet known, primary adult mammalian SC populations remain heterogeneous and at best enriched for SCs. This poses a major challenge toward the full characterization of these cells, given that impurities of the starting population in combination with destructive assays or discontinuous observation of the cells and their progeny can lead to inconclusive results.

Consequently, even more than 30 years after the determination of the fates of all cells in the development of the nematode *Caenorhabditis elegans* (Sulston et al., 1983), and despite major advances in more complex model organisms such as zebrafish (Keller et al., 2008), our knowledge of adult mammalian SCs and the genealogy of their lineages remains very limited. In addition, the integration of the molecular status of each developmental stage, which has recently been achieved in the nematode (Du et al., 2014), remains a major challenge for adult mammalian SCs.

A major bottleneck of SC research has been a lack of technologies to observe single SCs continuously over long times while noninvasively assessing the molecular status of single cells. The SC community has had to rely on population and/or snapshot readouts, which are insufficient to resolve the heterogeneity of SC populations (Figure 1A), capture the dynamics of molecules or phenotypes in a given cell (Figure 1B), or correlate current cellular states with future fates (Figure 1C). Recent technological improvements now provide us with tools to perform multiparameter single-cell measurements and single-cell manipulation, and some can even achieve this by tracking without killing the cells of interest (Table 1). In recent years, a plethora of molecular labeling tools for more efficient lineage tracing have also been developed. Continuous observation and tracking of single SCs and their progeny over time, the gold standard in studying SC systems, is continuously improving (Schroeder, 2011). Finally, approaches that allow acquisition of snapshot genomic, transcriptomic, and proteomic data from single cells are now becoming available.

Here, we review the current state of single-cell analysis for adult mammalian SCs and discuss how single-cell approaches are applied to answer key questions in SC research. We also provide examples of emerging fields and future challenges of single-cell analysis in SC research.

Cell Lineage Tracing

Cell lineage tracing aims to identify all progeny of a single cell in order to establish its lineage-differentiation and proliferation potential. This approach has been widely applied in developmental biology (Blanpain and Simons, 2013; Kretzschmar and Watt, 2012) and remains important to define SC properties in adult mammalian SC systems (Figure 2). As a minimal requirement, prospective adult SCs need to display longevity (i.e., the cell or its progeny need to persist throughout a large part of the lifetime of the organism) and lineage potential, which means that they can give rise to all cell types of the tissue they (re)generate. Lineage tracing can be performed by tagging a cell with a transmittable label or by continuous observation of the cell and all its progeny. The repertoire of tools to trace the fate of individual cells in vitro and in vivo has been updated by retroviral barcode vectors, multicolor reporter constructs, inducible recombinases, and the development of continuous live-cell imaging and tracking approaches.

Lineage Tracing In Vivo

Initial experiments on adult SCs were pioneered in the hematopoietic system by the works of Whitlock and Witte (1982), Weissman (Smith et al., 1991), and Mueller-Sieburg (Whitlock et al., 1987) in response to the groundbreaking discoveries of Till and McCulloch. They described the existence of hematopoietic cells in the bone marrow that can give rise to all types of blood cells and are able to self-renew (Becker et al., 1963). To show this, Becker et al. injected mouse bone marrow cells harboring different chromosomal abnormalities into recipient animals, which allowed them to identify the clonal origin of

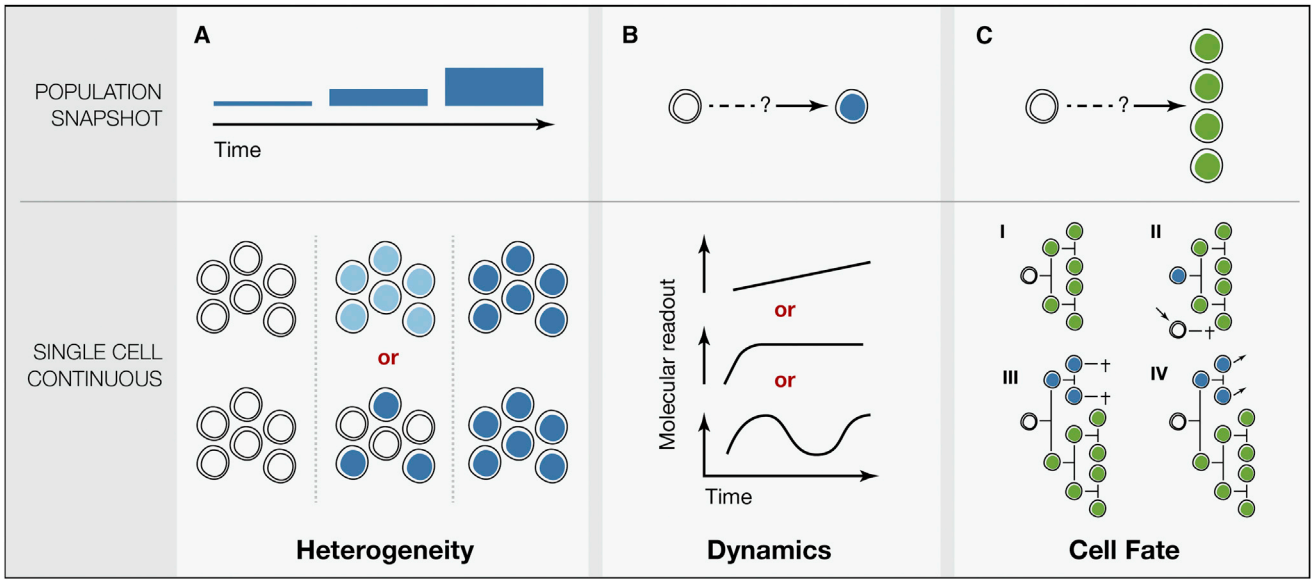

Figure 1. The Necessity of Continuous Single-Cell Analysis

(A) Population heterogeneity: bulk analyses mask the heterogeneity of a cell population. Only single-cell approaches reveal possible population heterogeneity (e.g., heterogeneous molecule expression, variable cellular behavior).

(B) Dynamics: only continuous observation of single cells reveals the dynamics of cellular properties changing over time.

(C) Cell fates in clonal dynamics: only continuous observation of single-cell fates allows nonambiguous conclusions about cell fate decisions underlying population outputs. Typical input/output analyses of dynamic cell systems produce snapshot data that can be interpreted by different conclusions about underlying cell fate choices. Here four very different (out of many more possible) conclusions about underlying cell fate choices are shown. Importantly, all four opposing conclusions are compatible with the observed snapshot data.

donor-cell-derived spleen colonies containing multiple hematopoietic lineages (Figure 2A) (Becker et al., 1963).

Lineage tracing is now an essential tool to study SC properties in adult mammalian tissues such as skin (Mascré et al., 2012), hair follicle (Claudinot et al., 2005; Rompolas et al., 2012), intestine (Barker et al., 2007), brain (Beckervordersandforth et al., 2010; Loulier et al., 2014), muscle (Rinkevich et al., 2012), bone (Park et al., 2012), and cancer (Youssef et al., 2010).

Single-Cell Transplantation

The gold standard approach to demonstrate SC function is single-cell transplantation (Table 2). In the blood system in vivo adoptive transfer experiments are routinely performed based on increasingly refined prospective isolation schemes for SC populations (Osawa et al., 1996; Sieburg et al., 2006; Yamamoto et al., 2013) (Figure 2B). Recently, Yamamoto et al. established an in vivo five-lineage tracing strategy to refine the HSC hierarchy after single-cell transplantation. They identified long-term self-renewing lineage-restricted myeloid/erythroid progenitors and proposed a myeloid-bypass model for the hematopoietic system (Yamamoto et al., 2013). Other examples for SC systems that have been studied using single-cell transplantation assays include muscle SCs (Sacco et al., 2008), hair follicles (Claudinot et al., 2005) and mammary SCs (Shackleton et al., 2006).

Clonal Tracing by Genetic Labeling

In vivo clonal lineage tracing requires tools to distinguish the transplanted cell and its progeny from the host. Various approaches in different cell systems have been developed to this end and have been reviewed recently in detail elsewhere (Blanpain and Simons, 2013). For the blood system congenic markers of two distinguishable alleles of CD45 (CD45.1 (Ly5.1)/CD45.2 (Ly5.2)) or Thy-1 (CD90.1/CD90.2) are routinely used. Using transgenic strains expressing a fluorescent protein or the LacZ gene for transplantation assays can also identify progeny of single cells. A novel labeling approach that has been widely applied is the introduction of genetic barcoding through lentiviral vectors (Gerrits et al., 2010; Lu et al., 2011; Naik et al., 2013, 2014; Verovskaya et al., 2013) (Figure 2C). This tool is an extension of clonality measurements based on the identification of unique genetic rearrangements, point-mutations, or deletions and retroviral integration site analysis by Southern blotting (Capel et al., 1990; Jordan and Lemischka, 1990). To perform clonal labeling, a heterogeneous population of cells is transduced with a lentiviral library coding for unique, "barcoded" DNA sequences. Each cell should receive a unique barcode, and after adoptive transfer, its progeny can be tracked by high-throughput sequencing. With this approach the clonal contribution of young and aged HSCs to blood generation was compared (Verovskaya et al., 2013). The result suggests that the individual clonal contribution to blood generation changes dynamically and that HSC pool sizes differ between old and young animals. In addition, skeletal distribution of HSC clones after adoptive transfer differed with age (Verovskaya et al., 2014). These results argue against previously suggested high-turnover rates of the HSC pool in vivo (Wright et al., 2001). The data further argue in favor of the notion that distinct hematopoietic niche microenvironments may be present in different skeletal bones. Another study using a similar approach demonstrated that hematopoietic reconstitution originates from a small pool of transplanted SCs in irradiated hosts, which can generate all cell types of the blood system (Naik et al., 2013). Paired transplantation of in vitro preamplified

Table 1. Modalities of Single-Cell Analysis Used in Stem Cell Research

	Lineage Tracing	Time-Lapse Imaging	Molecular Profiling
Approach	flow cytometry, sequencing, microscopy	microscopy	flow cytometry, mass cytometry, polymerase chain reaction, whole-genome and transcriptome sequencing, immunohistochemistry, fluorescence in situ hybridization
Condition	in vivo, in vitro	in vivo, in vitro	ex vivo
Parameters to be measured	phenotype of progeny, proliferation	single-cell fates (in vivo only short-term), proliferation, phenotype of progeny, interactions, motility, molecular dynamics	protein, DNA, RNA
Number of markers/ molecules	1–2	1–10	1–genome-wide
Destruction of cell upon measurement	depends on readout modality	no	yes
Temporal resolution	repeated readouts, endpoint analysis	continuous observation (in vivo <12 hr) and endpoint analysis	snapshot of single time point
Identification of cellular heterogeneity	yes	yes	yes
Full lineage tree	no	yes	no
Molecular dynamics	no	yes	no
Motility	no	yes	no
Interactions	no	yes	no

progenitors revealed a heritable lineage imprinting at early stages of HSC differentiation. Of note, a widespread criticism of adoptive transfer experiments and the ex vivo manipulation of SC is that such approaches do not recapitulate the steady-state behavior of SCs (Table 2) (Lu et al., 2011). To address this problem, a mouse line has been reported in which individual homeostatic HSCs are labeled with a unique genetic marker by transient induction of a transposase (Sun et al., 2014). The study suggests that not few HSCs, but rather a large number of lineage-committed, yet long-lived progenitors, contribute to steady-state hematopoiesis.

Recombinase-Based Lineage Tracing

Recombinase-driven genetic recombination has been widely applied to lineage tracing in many SC systems. To this end, a recombinase is expressed under a tissue-specific promoter to induce a conditional reporter gene. The recombination event permanently locks the expression of the reporter in the targeted cell type and its progeny. Two commonly used methods are the FLP-FRT and the Cre-loxP recombinase systems, of which the latter is most commonly used in mice. Here, a Cre-recombinase is expressed under a cell-type-specific or tissue-specific promoter. The Cre-recombinase expressing animals are crossed with a reporter line that contains a recombinase-inducible gene. To temporally restrict the activity of Cre-recombinase, inducible systems have been developed (Kretzschmar and Watt, 2012).

Cre-inducible reporter systems have been used in mice for instance to identify Lgr5, a marker for epithelial SCs (Barker et al., 2007); to foster the identification of prospective markers of adult neuronal SCs (Beckervordersandforth et al., 2010; Loulier et al., 2014); and to identify HSCs (Gazit et al., 2014).

Recently, variations of the recombinase-based labeling approaches have been developed to increase cell type specificity or the number of labels that can simultaneously be traced (reviewed in Kretzschmar and Watt, 2012). An important extension for single-cell lineage tracing in vivo is the use of multicolor Cre-inducible reporters. Applications include lineage tracing in the brain (Livet et al., 2007) and the intestine (Ritsma et al., 2014; Snippert et al., 2010).

A drawback of clonal fate mapping is that it often does not permit continuous observation of the steps that lead from the initial precursor to the differentiated progeny. Therefore it is not possible to study the proliferative history and molecular processes that occur during differentiation. These limitations can be overcome by continuous time-lapse imaging.

Time-Lapse Imaging

Continuous Live-Cell Imaging in Vitro

Continuous long-term live-cell imaging allows quantitative observation of single cells and all of their progeny over time for days to weeks (Okita et al., 2004; Schroeder, 2008, 2011). The required hardware for in vitro continuous single-cell imaging approaches is comparatively simple and would be in principle widely available in many cell biological laboratories. For the most part, video microscopes with a cell incubation unit, automated stages, and basic epifluorescence optics are sufficient. However, insufficient commercial software and the reliability of hardware components (such as automated focusing or mechanical shutters) still render most video-microscope setups useless for the continuous acquisition of sometimes weeks-long in vitro culture experiments. Data handling (currently on the order of terabytes), automated image processing, and software to track and quantify thousands of single cells across several days are additional challenges that currently hamper the widespread distribution of this technology. A detailed overview explaining the technical requirements for

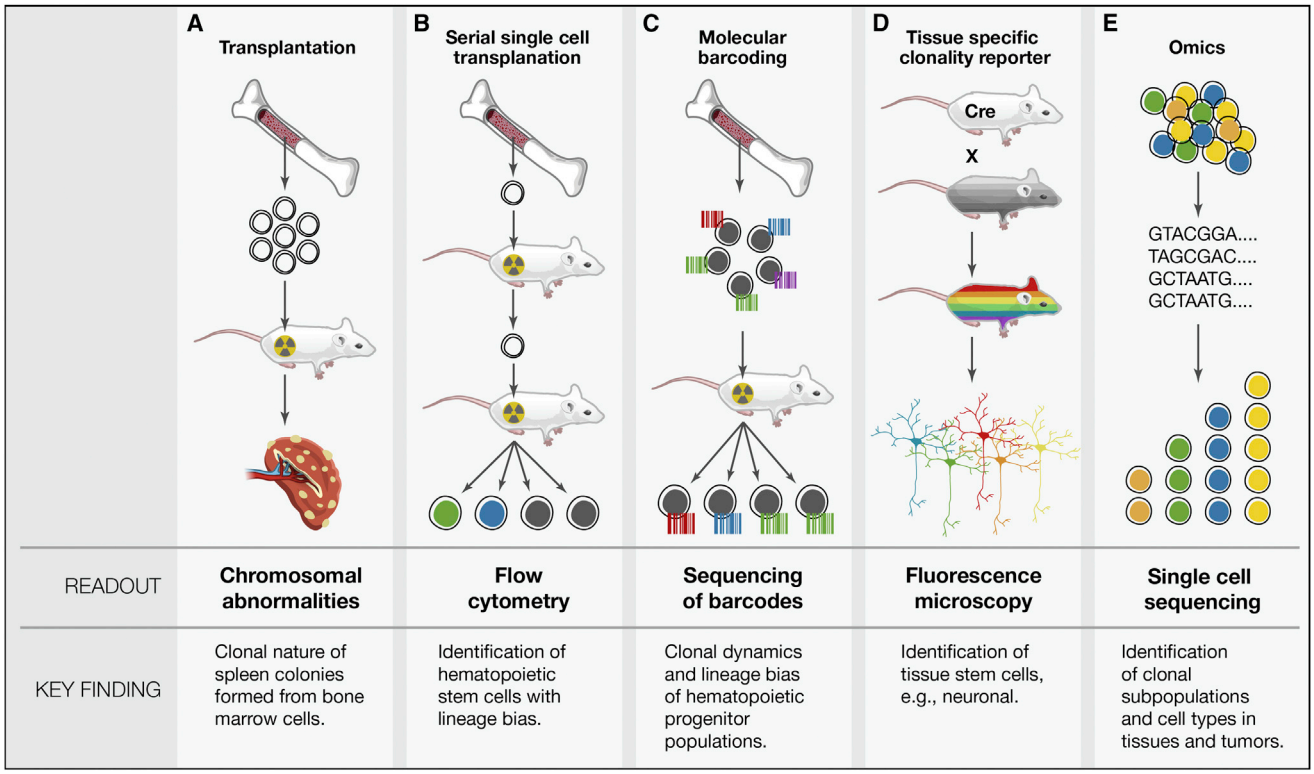

Figure 2. In Vivo Clonal Fate Profiling Approaches

(A) Transplantation of bone marrow cells leads to development of clonal spleen colonies. This experiment first demonstrated the capacity of bone-marrow-derived cells to generate hematopoietic cells of different lineages (Becker et al., 1963).

(B) Serial single-cell transplantation permits the study of the clonogenic and lineage potential and clonal bias in lineage production of HSCs (Osawa et al., 1996; Sieburg et al., 2006; Yamamoto et al., 2013).

(C) Molecular barcoding uses viral libraries with unique DNA-barcode sequences to label individual isolated hematopoietic progenitor cells, which permits the study of clonal dynamics and lineage bias in these cells (Lu et al., 2011; Naik et al., 2013; Verovskaya et al., 2013, 2014).

(D) Tissue-specific reporter expression can be achieved by crossing an inducible fluorescent reporter mouse with a mouse line expressing the inducer (e.g., Cre-recombinase) under a tissue-specific promoter. Random combinations of multiple fluorescent reporters in different cells can lead to dozens of possible clonal "colors" (Livet et al., 2007).

(E) Single cells isolated from tissues can be grouped by sequencing their transcriptome or genome to reconstruct their clonal relationships (Behjati et al., 2014; Jaitin et al., 2014; Abyzov et al., 2012; Frumkin et al., 2008; Navin et al., 2011).

in vitro time-lapse-imaging can be found elsewhere (Coutu and Schroeder, 2013).

Continuous imaging has proven to be a powerful tool to confirm the origins of hematopoietic cells from hemogenic endothelial cells (Eilken et al., 2009). In this study, continuous information on a cell's identity was combined with the continuous monitoring of cell morphology, cell adhesion, and several molecular markers. The results could later be confirmed by short-term in vivo imaging in developing zebrafish embryos (Bertrand et al., 2010; Kissa and Herbomel, 2010). Similarly, lineage analysis, tracking of cell morphology, and cell division frequencies were instrumental in understanding how key factors regulate neuronal development in the murine cerebral cortex (Asami et al., 2011; Costa et al., 2011). The unambiguous identification of each individual cell throughout differentiation was also crucial to reveal the instructive function of cytokines during hematopoietic lineage choice (Rieger et al., 2009). In this study, continuous cell fate analysis revealed that cytokines can instruct lineage choice, and not only support the survival of already lineage-restricted cells. A recent study applied continuous time-lapse imaging to identify cell cycle length as a mechanism regulating transcription factor concentrations (Kueh et al., 2013). Another example of this application is continuous time-lapse imaging to screen for normal development in human preimplantation embryos (Kirkegaard et al., 2013).

With an increasing set of transgenic reporter mouse strains and cell lines becoming available (e.g., Faust et al., 2000; Filipczyk et al., 2013) these tools should be exploited for functional studies in different SC systems. In addition, in-culture antibody staining approaches (Eilken et al., 2009, 2011), assays for live-cell RNA imaging (Lionnet et al., 2011; Ozawa et al., 2007; Strack et al., 2013), or approaches to monitor protein secretion profiles of single SCs have been reported (Zhao et al., 2014). Finally, endpoint analysis of intracellular protein (Gomez et al., 2013) or transcript expression (Lee et al., 2014) can complement continuous single-cell tracking.

Continuous Live-Cell Imaging in Vivo

Continuous noninvasive long-term observation of stem and progenitor cells and their progeny in their niche with high temporal

Table 2. Comparison of Single-Cell Approaches Used to Study Adult Mammalian SC Systems

	Prospective Cell Sorting and Transplantation	Live-Cell Imaging	Genetic Labeling Using Recombinase	Next-Gen Sequencing
Application	in vivo (but including ex vivo purification step)	in vitro and in vivo	in vivo	in vivo
Strengths	multiparameter flow cytometry based sorting; progeny clearly identifiable (dye, viral labels, barcoding, allelic markers)	continuous observation throughout several days (in vitro only); complete genealogy; molecular dynamics	no disruption of in vivo context (niche)	no disruption of in vivo context (niche); no labeling required; genome-wide information
Weakness	ex vivo purification (and labeling) may affect viability and development; invasiveness of transplant; potentially heterogeneous populations, if no single-cell transplant; insufficient prospective markers; no genealogy; "snapshot" type of readout	in vitro culture may not reflect in vivo conditions; no continuous in vivo imaging for continuous readout of long-term fate; requires prospective isolation of enriched (pure) SC populations	lack of SC-specific promoters; often restricted to a single marker (two possible); no genealogy (except multicolor labeling); requires generation of transgenic reporter animals; "snapshot" type of readout	no combination of genome and transcriptome information (yet); no dynamics; technical limits toward number of cells being probed (to date); "snapshot" type of readout
Examples of tissues to which methods were applied	blood, muscle, skin, mammary gland	blood, neurons, skin, pancreatic islets, intestinal crypts, muscle	blood, neurons, skin, intestinal crypts, bone, any tissue with a known specific promoter	blood, cancer

and spatial resolution would be optimal for understanding SC biology. Currently, live-cell in vivo imaging and tracking of single cells is successfully applied in the developmental biology of non-mammalian embryos. Here, imaging tools for the visualization from a single cell to a whole multicellular organism have been reported (reviewed in Höckendorf et al., 2012). However, live-cell imaging with sufficient spatial and temporal resolution of adult SCs in mammals poses major technical challenges and remains restricted to extremely few specialized cases. Despite major advances in noninvasive biomedical imaging modalities such as MRT, microCT, or fluorescent molecular tomography (Nguyen et al., 2014), such techniques still lack sufficient spatial resolution to trace single cells in vivo and map their fates over time. The only widespread available live-cell imaging modalities for in vivo cell tracing thus are multiphoton- and confocal light-microscopy (Pittet and Weissleder, 2011; Schroeder, 2008). These imaging modalities, however, lack sufficient penetration depth for direct noninvasive imaging of most adult SC niches. In addition, they only allow observation of very small areas, requiring immobilization and surgical exposure of tissues. This poses a major hurdle for meaningful in vivo single SC fate mapping approaches because the maximum tolerable time an animal can be kept alive under anesthesia on the microscope stage is limited (usually restricted to 6–12 hr). Especially for SC systems with low SC frequency, long divisional rates, and heterogeneity such as in the hematopoietic system, it is impossible to observe sufficient SC divisions and cell phenotype changes during such short intervals. Moreover, in particular for HSCs, SC niches are poorly defined, and in contrast to highly organized endothelial tissues, clear orientation marks to retrace cells during repeated imaging sessions are mostly absent. Thus, to date, intravital imaging studies on the hematopoietic system have been restricted to short-term, mostly immunity-related aspects of hematopoiesis (Massberg et al., 2007).

Notwithstanding these technical challenges, intravital confocal imaging and multiphoton imaging have been successfully applied to study several adult SC systems. Skin and hair follicles are readily accessible for intravital microscopy and allow extended imaging and tracing over repeated imaging sessions. In one study, the epithelial SC niche was characterized using noninvasive labeling with a fluorescent protein in vivo (Tumbar et al., 2004). In another study, GFP-labeled SCs of the hair follicle were traced, and the interaction of cells with neighboring supportive mesenchymal cells was mapped during hair follicle regeneration (Rompolas et al., 2012). The murine testis represents another easily accessible tissue. Two studies applied wide-field fluorescence time-lapse microscopy to track the SC pool that provides steady-state spermatogenesis in surgically exposed murine testis. This work revealed the nonhomogenous composition of the SC pool that supports spermatogenesis and the continuous replacement of spermatogenic SCs. The intestinal epithelium is constantly regenerated by crypt base columnar (CBC) SCs that are located around the base of the intestinal crypt and express Lgr5 (Barker et al., 2007). Whether CBC SCs are equally capable of contributing to self-renewal and differentiation has been a much-debated question. Using multiphoton microscopy and an abdominal imaging window, Ritsma et al. have applied time-lapse imaging to the intestinal crypt in vivo (Ritsma et al., 2014). Multicolor clonal labeling facilitated tracing individual SC progeny restricted to Lgr5 expressing-intestinal SCs. This study demonstrated that within the heterogeneous population of intestinal SCs, each SC is able to function equally as a long-term contributor to self-renewal and differentiation.

Single-Cell Molecular Profiling

Until recently, molecular profiling techniques for probing a cell's proteome, transcriptome, or genome have not been sensitive enough to be applied at single-cell resolution. This issue is

Table 3. Single-Cell Molecular Profiling Approaches

	FISH	qPCR	ddPCR	DNA-seq	RNA-seq	Proteomics and Flow Cytometry	Continuous Live-Cell Imaging
Instrumentation	fluorescence microscope	Fluidigm C1, qPCR reader	Biorad ddPCR droplet generator and reader	Illumina, Ion Torrent	Illumina, Ion Torrent, SOLiD	flow cytometry, CyTOF, microfluidic platforms	fluorescence microscope (epi/ confocal)
Number of markers/ molecules	≦32	≦300	≦10	genome-wide	genome- wide	≦50	≦10
Destructive	yes	yes	yes	yes	yes	yes/ no	no
Temporal resolution	snapshot	snapshot	snapshot	snapshot	snapshot	snapshot	continuous and snapshot
References	(Liu et al., 2009) (Lubeck & Cai, 2012)	(Guo et al., 2010) (Moignard et al., 2013) (Guo et al., 2013a)	(Warren et al., 2006),	(Hou et al., 2013) (Navin et al., 2011)	(Tang et al., 2010) (Yan et al., 2013) (Faddah et al., 2013) (Jaitin et al., 2014)	(Yamamoto et al., 2013) (Kemper et al., 2012) (Ludin et al., 2012) (Bendall et al., 2011) (Bendall et al., 2014).	(Eilken et al., 2009) (Rieger et al., 2009) (Kueh et al., 2013) (Rompolas et al., 2012) (Tumbar et al., 2004) (Klein et al., 2010; Nakagawa et al., 2010) (Ritsma et al., 2014)

problematic because bulk assays mask heterogeneity at the single-cell level that could have future (fate) consequences for the cells being observed (Figure 1A). With the help of improved sample preparation, for instance through microfluidic devices, insensitivity and cost have been significantly reduced, which now makes it feasible to perform single-cell molecular profiling of many cells with readouts ranging from dozens of components to genome-wide coverage (Table 3). However, most molecular profiling approaches are destructive, thus providing only a snapshot view of a molecular status of a cell (Figure 1), which cannot be correlated with future fates of the cells studied. This caveat is a problem in particular when SC populations can be only enriched to insufficient purity. In this section we discuss the current state of single-cell molecular profiling approaches and refer to examples of SC-relevant applications whenever possible.

Flow Cytometry

Flow cytometry is the most widely applied single-cell method for characterization and, in combination with cell sorting, isolating SCs. Flow cytometry detects cellular parameters such as size, morphology, cell cycle phase, or DNA content for a large number of cells. Fluorophore-conjugated antibodies against intracellular or extracellular markers allow detection of proteins and their activation status (e.g., phosphorylation). Fluorescence-activated cell sorting (FACS) further permits selective isolation of intact, living single cells, which makes this technology crucial for many clonal applications and downstream assays of single SC analysis. In the hematopoiesis field flow cytometry has been instrumental in determining prospective markers of rare HSCs in the bone marrow (Kemper et al., 2012; Osawa et al., 1996; Spangrude et al., 1988) and continues to be a key technology for single-cell studies on SCs (Yamamoto et al., 2013).

The parameters that can be detected by multicolor flow cytometry are ultimately limited by the number of fluorescent dyes that can be spectrally resolved. To overcome this limitation next-generation cytometry techniques have been developed. Image-stream cytometers acquire spatially resolved pictures of cells passing through the flow cytometry instrument. Adding spatial information to cytometry can be used to define intracellular localization of proteins or discern morphological features of a large number of single cells. This approach has been used to identify a subset of myeloid cells in the bone marrow that interacts with HSCs and induces their upregulation of COX2 under stress (Ludin et al., 2012). Recently a new generation of flow cytometers with improved spectral resolution, enabling "hyperspectral cytometry," has been introduced to the market. The new instruments contain a multiprism monochromator that replaces the beam splitters and filters of conventional cytometers. This way signal loss is minimized and detection up to 15 spectrally resolved colors with only two lasers becomes possible (Grégori et al., 2014). Furthermore the introduction of disposable microfluidic cartridges that replace traditional quartz flow cells reduces liquid throughput and permits sorting of cells at far lower pressure than before, which improves cell survival. Improvements in microfluidics sorting approaches and their combination with high-throughput microscopy and image analysis approaches will likely compete with and eventually replace the very costly traditional flow cytometers and sorters that are currently in use (Mazutis et al., 2013).

Mass Cytometry and High Dimensional Single-Cell Imaging

Inductively coupled plasma mass spectrometry ("CyTOF") enables an even greater amount of components to be studied at a time from a single cell. To this end, antibodies are labeled with isotopes of rare-earth elements instead of fluorophores. State-of-the art technology now claims to simultaneously resolve >100 individual probes at a time. This technology has been applied to study the hematopoietic system and the immune system by dissecting the responses of hematopoietic progenitor cells toward small-molecule-based perturbation (Bendall et al., 2011). The study revealed heterogeneity among previously considered homogenous cell populations but also highlighted unexpected overlapping signaling responses across distinct populations of the hematopoietic system. Another study was

able to reconstruct a developmental hierarchy of signaling events during B lymphopoiesis (Bendall et al., 2014).

A further extension of mass cytometry is its application for reading protein expression signatures from intact tissue sections. This approach permits one to analyze IHC samples stained with metal-conjugated antibodies in a rasterized fashion directly in a mass cytometer. In contrast to flow cytometry, which requires samples in suspension, the spatial integrity of the tissue sections can be maintained and images reminiscent of microscope images of tissue sections are reconstructed (Angelo et al., 2014; Giesen et al., 2014). In line with this technique, another compound approach termed array tomography relies on the serial acquisition of high-resolution fluorescent microscopy and backscatter-electron microscopy images of ultrathin tissue sections. Subsequently images are computationally reconstructed using data of both modalities (Micheva and Smith, 2007).

Multiparameter imaging technologies will be of importance in the SC field, for instance to dissect SC niches such as the bone marrow niche of HSCs where current immunofluorescent imaging approaches reach the technical limits of resolvable fluorophore combinations. Despite their great potential, the technologies however remain far from routine application. This paucity is mainly due to the current lack of commercially available software for data analysis, commercially available rare-earth-labeled antibodies, and high hardware costs.

Single-Cell Proteomics

Single-cell proteome studies are currently challenging due to the lack of sufficiently sensitive instrumentation for proteome-wide readouts in single cells. As readouts, the aforementioned fluorescence and mass cytometry tools represent the most powerful options (Bendall et al., 2012). This superiority is because by far the highest degree of multiplexing for multiprotein expression profiling in single cells can be achieved today by the use of protein-specific antibody probes. Other highly sensitive and versatile techniques that can be applied to single cells are proximity ligation assays (PLAs). Antibody-DNA conjugates are used to bind target molecules and are amplified using a rolling-circle polymerase chain reaction (PCR) (Weibrecht et al., 2010). By using two independently binding antibody-DNA conjugates targeted against the same protein, PLA ensures high specificity and potentially single-molecule resolution. Furthermore microfluidic-based proteome chips have been reported, permitting quantitative yet still low-scale multiplexed detection of proteins (Willison and Klug, 2013). A drawback when using antibody probes is their variable specificity. To improve detection even with nonspecific antibodies at hand a single-cell western blotting method has been developed that first separates the protein content of single cells by its molecular weight in a miniaturized type of a polyacrylamide gel prior to antibody-based detection. This approach was reported to resolve up to 11 proteins in 1,000 independently assayed single cells on a single microscope slide (Hughes et al., 2014). Finally one study generated protein fusions with a fluorescent protein to screen the expression dynamics of 93 genes in the nematode *C. elegans* (Liu et al., 2009). However, tagging approaches may affect protein function and the implementation of this approach for a large number of genes in other SC systems remains technically challenging.

PCR

PCR is a sensitive method to amplify genomic DNA and to quantify mRNA and microRNA transcripts after reverse transcription. The power of PCR for genomic single-cell analysis was recognized early and was pioneered in the SC field, e.g., for haplotype analysis of human sperm (Li et al., 1988) and the subcloning and detection of transcripts in single hematopoietic progenitors (Brady et al., 1990). Quantitative reverse transcription PCR (RT-qPCR) can be used to measure mRNA levels. Integration of RT-qPCR into microfluidic chips and single droplet assays now facilitates multiplexed and quantitative applications at the single-cell level. Commonly, after reverse transcription, a preamplification step using multiplexed primers targeted against the genes of interest is performed. In this way the cDNA obtained from a single cell can be used to quantify the expression of up to 100 transcripts (White et al., 2011). Alternatively, digital PCR (ddPCR) provides an absolute count of a transcript of interest (Guo et al., 2012). The cDNA product of a single-cell reverse transcription reaction is split into very high numbers of little compartments (“droplets”), such that each contain either one or no cDNA molecule of interest. The number of compartments with PCR product then equals the number of individual transcripts of the given gene.

A limitation of PCR is the low number of genes that can be studied at a time. This restriction makes this approach biased with respect to the choice of candidate genes. Despite this limitation, targeted single-cell screens have been shown to be instrumental in revealing heterogeneity in SC populations. For instance, cell lineages of the murine embryo could be reconstructed based on gene expression signatures of key developmental transcription factors (Guo et al., 2010). Another study used a targeted single-cell transcriptional profiling approach probing 77 genes for their association with Nanog downregulation in embryonic SCs (ESCs). The single-cell expression signatures confirmed upregulation of differentiation and cell-cycle-associated genes in response to Nanog loss (MacArthur et al., 2012). A similar strategy was used to study gene expression networks in hematopoietic stem and progenitor cells (Moignard et al., 2013). Quantification of 18 lineage-specifying transcription factors revealed a new regulatory relationship among three master regulators of hematopoietic cell fate. The signatures also allowed progenitor populations to be distinguished by selectively expressed sets of key transcription factors. In another study, an expression panel of 280 cell surface markers was used to reveal heterogeneity among the progenitor populations of the hematopoietic system (Guo et al., 2013a). The study found distinct subsets among purified hematopoietic precursors and it was possible to computationally reconstruct a map of the cellular hierarchy underlying hematopoiesis.

ddPCR has been used to quantify the abundance of PU.1, a major regulator in hematopoiesis, in individual hematopoietic stem and progenitor populations (Warren et al., 2006). ddPCR has also been used to provide an absolute count of genetic diversity within a given population of cells. In a study on human-fibroblast-derived induced pluripotent SCs (iPSCs), it was demonstrated that somatic cells in an adult human individually harbor a significant amount of acquired somatic copy number variations. This finding proves the notion that somatic cells are

not identical, but instead display a significant degree of mosaicism (Abyzov et al., 2012).

Single-Cell Sequencing

Single-cell genomic and transcriptomic sequencing are powerful new technologies with great potential in the SC field. The ability to reach single-cell sensitivity has relied upon the development of protocols that provide sufficient amplification with minimal amplification bias in order to reach whole-genome coverage. Several protocols have been developed to this end for single-cell-derived DNA and RNA sequencing. In-depth reviews on the technical details and differences among these approaches have been recently published (Junker and van Oudenaarden, 2014; Shapiro et al., 2013).

Single-Cell Genome Sequencing

Seemingly identical cells in an adult organism may acquire differences in their genomes due to dynamic changes in their DNA. Single-cell genome sequencing can be used to resolve such differences. By using a DNA amplification method that ensured a high genome coverage (MALBAC), it was possible to detect copy number variations and single-nucleotide polymorphism (SNP) among the genomes of three individual cells (Zong et al., 2012). Another aspect of single-cell genomics is the potential to detect genome rearrangements, for instance occurring during mitosis. To this end two studies used whole-genome sequencing to analyze the genomes of human sperm at single-cell resolution (Lu et al., 2012; Wang et al., 2012). By comparing the genome sequences obtained from single sperm with the donor's genetic codes, both studies were able to establish de novo mutation rates and determine chromosomal recombination frequencies. Lu et al. further reported that autosomal aneuploidy correlated with an overall lower incidence of crossovers in sperm. In a similar study, sequencing of both polar bodies of human oocytes was used to derive information about aneuploidy or SNPs in disease-associated alleles, while retaining the oocyte otherwise intact (Hou et al., 2013). Single-cell genome sequencing can also be used to derive structural information about the nuclear architecture. Nagano et al. used single-cell genome sequencing to obtain a chromosomal contact map of crosslinked DNA. The crosslinking patterns were used to reconstruct chromosomal territories within the nucleus (Nagano et al., 2013). Finally, Guo et al. have performed methylome analysis at the single-cell level. They applied this approach to detect CpG islands within the genome of single mouse ESCs (mESCs), haploid sperm cells, and mouse zygote pronuclei (Guo et al., 2013b).

Single-Cell Transcriptome Sequencing

Similar to single-cell genome sequencing, single-cell RNA sequencing (RNA-seq) provides a highly resolved picture of the transcriptome of a single cell. This data can be of particular interest in cell populations of differentiating cells where transitions from one cell type to another likely are reflected by the underlying changes in transcription. Tang et al. used single-cell sequencing to determine the transcriptional changes, including the changes in transcript variants, negative regulators of transcription such as microRNAs, and epigenetic regulators during the derivation of ESCs from the inner cell mass (Tang et al., 2010). RNA-seq can also be applied to reveal differences among seemingly identical cell populations and draw conclusions on their genomic regulation. In a study that compared the response of a population of bone-marrow-derived dendritic cells (BMDCs) toward a challenge with lipopolysaccharides (LPS), the observed heterogeneous response at single-cell transcriptome level could be attributed to differences in maturation stages of the cells in the in vitro culture system (Shalek et al., 2013). Pairwise comparisons yielded clusters of genes that share similar function. In a follow-up study, more than 1,700 single cells were exposed in a highly combinatorial microfluidic approach to a set of conditions mimicking a pathogen infection (Shalek et al., 2014). Cells were kept either in isolation or in coculture for paracrine signaling. Interestingly, cell-to-cell variability in a primary inflammatory gene-module decreased in cells that were not permitted to exchange paracrine signals. Another study aimed to use RNA-seq to determine the incidence of monoallelic expression of transcripts in single cells. This phenomenon has been thought to be restricted to a few cells during early embryonic development and to be transmitted to adult cells. Deng et al. used RNA-seq to study allelic expression patterns of mouse preimplantation embryos (Deng et al., 2014). Monoallelic expression of autosomal genes was reported in 12%–24% of transcripts in both embryonic and differentiated cells. In departure from the concept that monoallelic expression is an inherited trait established during embryonic development, the study suggests that monoallelic expression can appear in a random fashion in embryonic and adult cells. The fact that expression from heterozygous alleles occurs in a variable fashion will be important to explain susceptibility to genetic disorders. A variation of single-cell RNA-seq that relies on mRNA capture by photoactivation was used to capture mRNAs from selected single cells in complex tissues such as the brain in situ (Lovatt et al., 2014).

Single-Cell RNA FISH

Single-cell RNA fluorescence in situ hybridization (FISH) allows the absolute quantification of mRNA transcripts in single cells by specific hybridization of fluorescently labeled probes to target RNA molecules (Junker and van Oudenaarden, 2014). Single-cell RNA FISH and single-cell qPCR was used in combination in a candidate approach to enable the study of the variability of gene expression during the reprogramming process of iPSCs (Buganim et al., 2012). Since single-cell RNA FISH allows one to determine absolute molecular counts of mRNA, this approach can also serve to validate whole-transcriptome sequencing data (Liu et al., 2009). In combination with super resolution microscopy and the combinatorial use of fluorophores to label mRNAs in a spatially defined manner, the detection of up to 32 mRNAs at a time in a single cell has been reported (Lubeck and Cai, 2012).

Next Steps and Future Challenges for Single SC Studies

An increasing number of single-cell technologies now allow us to address longstanding biological problems and challenge existing paradigms in SC biology. Nevertheless, technical limitations still exist that need to be overcome in order to make full use of single-cell approaches in SC biology. Below we highlight several of those areas that we believe will be of future importance to the SC field.

Single Sequencing for Lineage Tracing

An exciting aspect of single-cell sequencing is that it has a large potential to be exploited for single-cell lineage tracing in an entirely label-free manner. Until recently, the number of cells that can be processed within reasonable time and cost has limited such approaches. Microfluidic technology for

high-throughput sample preparation, decreasing sequencing costs, and the establishment of precise technologies for accurate molecule counting in sequencing data now make such approaches feasible. Several studies have already made use of single-cell whole-genome sequencing for unbiased clonal cell tracing (Figure 2E). In a study on human breast cancer, single-cell genome sequencing has been used to detect copy number variations in individual cells and to identify clonal populations of tumor cells that correspond to different waves of tumor expansion (Navin et al., 2011). More recently a similar approach was applied in clonal cell lines derived from nonmalignant murine tissues (Behjati et al., 2014). The sequences obtained from these lines were analyzed with respect to their number and quality of mutations, which could be correlated with the divisional history and type of tissue the cells were derived from. Furthermore, the approach allowed the estimation of the contribution of embryonic precursors to adult tissue cells. Also, single-cell exome sequencing can serve as a basis for delineation of clonal relationships (Xu et al., 2012) or the dissection of complex tissues into distinct cell types. In a proof-of-principle study of a population of splenocytes enriched for the dendritic cell (DC) marker CD11c, more than 4,000 single cells were analyzed in this manner (Jaitin et al., 2014). A critical component of this study was a newly developed sequencing protocol (MARS-Seq) that ensured accurate molecule counting of the single-cell transcripts. Four groups of gene modules that correlate with known expression signatures of different subtypes of cells in the immune system could be clustered. When CD11c-enriched cells were isolated after an LPS challenge of the animals, a different composition of the DC subpopulations could be observed. Notably, the authors report a large variability with respect to the expressed genes. Such variability may be indicative of the existence of previously not known, differentially responding subsets of a given cell type.

Monitoring of Cell-Extrinsic and Cell-Intrinsic Cues

Signaling events triggered by extrinsic cues such as growth factors, cytokines, or signals from the microenvironment influence the outcome of cell differentiation (Endele et al., 2014; Rieger et al., 2009). Receptors for different stimuli, although supporting different cell fates, frequently activate several identical signaling pathways. The cellular response of a given cell stimulated with a particular extrinsic signal is determined by the integration of the crosstalk of several synchronously or sequentially activated signaling pathways and their dynamics and strength. Moreover, even in seemingly homogenous cell populations, single cells can respond heterogeneously to a particular external stimulus, which can be linked to different future cellular behavior (Figure 1A) (Tay et al., 2010). Consequently, methods are required that allow the detection and quantification of the temporal dynamics of signaling pathway activities with single-cell resolution in live cells. Additionally, since initial signaling events and eventual cell fate decisions can be temporarily separated by days, detection of signaling needs to be combined with methods that allow tracking future fates of single cells. The latter can be achieved by long-term time-lapse imaging, as discussed above, or by single live-cell isolation and subculture after observing signaling responses. To this end manipulation, for instance by microfluidic platforms that allow one to individually address, select, and isolate single cells of interest, will be required.

A variety of biosensors based on fluorescence that allow the detection and quantification of signaling dynamics or intermediate signaling components (e.g., second messengers) in live cells has been described. Subcellular translocation events of signaling components are a recurring feature of many activated signal transduction pathways. Fusion of the protein (full length) or the protein domain responsible for translocation to a fluorescent protein visualizes these events and can therefore be used to detect pathway activity. Using a p65-GFP fusion protein, this technique has, for example, been exploited to study the dynamics of NFκB signaling in single cells (Tay et al., 2010). Other fluorescent biosensors for signaling activity are based on Förster resonance energy transfer (FRET). FRET-capable donor and acceptor fluorophores can either be tagged to separate proteins (intermolecular FRET) or exist within one polypeptide (intramolecular FRET). In the latter, the modification of the sensor by a signaling pathway of interest results in conformational change of the sensor and in FRET. Because this is a reversible process, signaling dynamics can be measured over time. FRET-based sensors have been developed for a variety of different pathways, as reviewed elsewhere (Newman et al., 2011).

Studying signaling events also requires their precise spatial and temporal manipulation. New tools for signaling perturbation, such as light-inducible systems (Toettcher et al., 2011), in combination with sensors for signaling activity will allow important new insight into how specific signaling components and their temporal coordination are involved in orchestrating SC decisions (Figure 1B). For an in-depth review of the topic the reader is referred to Purvis and Lahav (2013).

Improving In Vitro Observation, Culture, and Manipulation of Single SCs

To date, long-term continuous observation of SCs and their progeny is limited by the lack of suitable out-of-the-box time-lapse microscopes including required incubation and manipulation devices and software. Currently, scientists still have to build their own instrumentation because commercial suppliers of microscopes continue to fail to provide adequate compound solutions.

Advances in microfluidic, microdroplet, and hanging-drop technologies point the way toward an entirely different, "programmable" approach of in vitro culture systems that will permit parallel readouts for thousands of single cells in one experiment (Frey et al., 2014; Gómez-Sjöberg et al., 2007). For instance, spatial confinement of individual cells in arrayed compartments makes it feasible to assess direct functional outcomes of intracellular signaling and to assay the dynamics of their interactions (Tay et al., 2010), secretion profiles (Zhao et al., 2014), and their clonal fate (Lecault et al., 2011). Furthermore, intracellular signaling processes can be studied at single-cell resolution by avoiding paracrine effects of neighboring cells, as is the case in bulk cultures. Rare cell populations can be profiled at a proteomic level through microfluidic antibody capture (Willison and Klug, 2013) and combining microfluidics with optical tweezing enables isolation of preselected single cells from culture systems in order to do quantitative single-cell readouts or further clonal analysis (Landry et al., 2013).

Such approaches will be particularly important to make full use of human patient-derived iPSC models. Chemical compound screens, or large-scale genetic alterations through short hairpin

RNAs (shRNAs) or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) (Holt et al., 2010) in functional screens, will require massive miniaturization and parallelization of cell culture approaches (de Souza, 2014). At the same time, efforts are ongoing to create artificial niches with the aim to provide cues that foster SC maintenance or the directed differentiation into select tissues (Lutolf et al., 2009; Torisawa et al., 2014).

Biological Uncertainty and Computational Solutions

A critical disconnect exists between the now available single-cell molecular profiling tools and in vivo or in vitro continuous fate mapping approaches. With the exception of continuous time-lapse imaging, single-cell molecular profiling studies to date require destruction of the cell being investigated. This requirement renders it impossible to correlate the consequences of a given molecular constitution with the future fate of the cell. On the other hand, continuous, noninvasive observation or in vivo fate mapping approaches only permit a few molecular markers to be analyzed at a time. Additionally, snapshot analysis of single cells at transcriptome-wide levels bears the risk of overestimating cellular heterogeneity. RNA expression, for instance, occurs in bursts, thus even cells with identical gene expression will display the same set of mRNA at different levels at a given time point. Over time, however, a given cell will average these effects, as seen in bulk population based analysis. It will be key to study, at the single-cell level, whether such fluctuations of gene expression over time indeed induce alternate cell fate choices in SCs, as has been previously suggested (Enver et al., 1998), and how these fluctuations potentially modulate responses to cell-extrinsic instructive cues received by a cell. However, given the current destructive approaches to measuring gene expression, future solutions are yet to be found to dissect such effects in intact cells and relate them to clonal cell fates. A solution to this problem might be the use of continuous in vitro observation of single cells and their progeny in order to empirically build a deterministic model of cell states at any time during the observation. Reoccurring patterns can then be used to identify and computationally predict future cellular behavior (Cohen et al., 2010). Single cells could then be isolated at key time points, for instance briefly before or after a predicted cell division, to reveal the underlying molecular constitution at critical time points. With such tools at hand, future efforts should focus on combining multiple types of single-cell whole-genome, transcriptome, and proteome readouts.

Clinical Application of Single SC Analyses

Single-cell approaches with functional readouts of SCs in vitro and in vivo have been proven to be important for regenerative and reproductive medicine (Nguyen et al., 2014). However, most of the approaches to monitor, for instance, transplanted SCs in humans do not yet permit single-cell resolution. One of the first routine applications of single-cell analysis is in reproductive medicine. Next-generation sequencing approaches are used in preimplantation diagnostics and quality control for in vitro fertilization (Wells, 2014). The technology is used in screenings for preimplantation genetic diagnosis (PGD) of aneuploidy to select chromosomally normal embryos. In addition, it holds the promise to identify traits that lead to improved pregnancy rates, reduced miscarriage, or reduced chromosomal defects (Wells, 2014). Another technology used in preimplantation diagnostics is time-lapse microscopy, which permits continuous and noninvasive monitoring of the germinal stage during the first days after in vitro fertilization. A study correlated time-lapse imaging data of human embryos with gene expression analysis and provided evidence that characteristic behaviors of the embryo during the first cell divisions toward the three- and four-cell stage reflect underlying molecular processes of embryonic development (reviewed in Kirkegaard et al., 2013). Continuous observation of human preimplantation embryos in clinical time-lapse incubators now permits identification of embryos with normal behavior to predict further success of implantation and development (Kirkegaard et al., 2013). With increasing appreciation of clonal heterogeneity in cancer, such approaches could be also used to dissect distinct cancer (stem) cell lineages in leukemia or solid tumors. These analyses will enable improved targeted therapy and may ultimately help to identify traits that lead to tumor relapse or tumor immune evasion and develop strategies to address these problems therapeutically.

Summary

New molecular readouts and functional screening tools are constantly being developed for single-cell analyses. The new methods are beginning to complement traditional experimental strategies and have already been used to answer several long-standing questions of SC biology. As often is the case with novel technologies, it is important to bear in mind technical limitations, unexpected effects, and an overinterpretation of results (Germain, 2014). While (continuous) single-cell quantification will ultimately be required for a complete understanding of many processes, it will not always be required or be the most informative approach for a given question. Eventually, the integration of quantitative single-cell analysis with computational prediction and high-throughput in vitro and in vivo approaches will allow deciphering fates of SC systems and ultimately the ontogeny of all tissues.

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REFERENCES

Abyzov, A., Mariani, J., Palejev, D., Zhang, Y., Haney, M.S., Tomasini, L., Ferrandino, A.F., Rosenberg Belmaker, L.A., Szekely, A., Wilson, M., et al. (2012). Somatic copy number mosaicism in human skin revealed by induced pluripotent stem cells. Nature *492*, 438–442.

Angelo, M., Bendall, S.C., Finck, R., Hale, M.B., Hitzman, C., Borowsky, A.D., Levenson, R.M., Lowe, J.B., Liu, S.D., Zhao, S., et al. (2014). Multiplexed ion beam imaging of human breast tumors. Nat. Med. *20*, 436–442.

Asami, M., Pilz, G.A., Ninkovic, J., Godinho, L., Schroeder, T., Huttner, W.B., and Götz, M. (2011). The role of Pax6 in regulating the orientation and mode of cell division of progenitors in the mouse cerebral cortex. Development *138*, 5067–5078.

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., and Clevers, H. (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature *449*, 1003–1007.

Becker, A.J., McCulloch, E.A., and Till, J.E. (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature *197*, 452–454.

Beckervordersandforth, R., Tripathi, P., Ninkovic, J., Bayam, E., Lepier, A., Stempfhuber, B., Kirchhoff, F., Hirrlinger, J., Haslinger, A., Lie, D.C., et al. (2010). In vivo fate mapping and expression analysis reveals molecular

hallmarks of prospectively isolated adult neural stem cells. Cell Stem Cell *7*, 744–758.

Behjati, S., Huch, M., van Boxtel, R., Karthaus, W., Wedge, D.C., Tamuri, A.U., Martincorena, I., Petljak, M., Alexandrov, L.B., Gundem, G., et al. (2014). Genome sequencing of normal cells reveals developmental lineages and mutational processes. Nature *513*, 422–425.

Bendall, S.C., Simonds, E.F., Qiu, P., Amir, A.D., Krutzik, P.O., Finck, R., Bruggner, R.V., Melamed, R., Trejo, A., Ornatsky, O.I., et al. (2011). Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. Science *332*, 687–696.

Bendall, S.C., Nolan, G.P., Roederer, M., and Chattopadhyay, P.K. (2012). A deep profiler's guide to cytometry. Trends Immunol. *33*, 323–332.

Bendall, S.C., Davis, K.L., Amir, A.D., Tadmor, M.D., Simonds, E.F., Chen, T.J., Shenfeld, D.K., Nolan, G.P., and Pe'er, D. (2014). Single-cell trajectory detection uncovers progression and regulatory coordination in human B cell development. Cell *157*, 714–725.

Bertrand, J.Y., Chi, N.C., Santoso, B., Teng, S., Stainier, D.Y.R., and Traver, D. (2010). Haematopoietic stem cells derive directly from aortic endothelium during development. Nature *464*, 108–111.

Blanpain, C., and Simons, B.D. (2013). Unravelling stem cell dynamics by lineage tracing. Nat. Rev. Mol. Cell Biol. *14*, 489–502.

Brady, G., Barbara, M., and Iscove, N.N. (1990). Representative in vitro cDNA amplification from individual hemopoietic cells and colonies. Methods Mol. Cell. Biol. *2*, 17–25.

Buganim, Y., Faddah, D.A., Cheng, A.W., Itskovich, E., Markoulaki, S., Ganz, K., Klemm, S.L., van Oudenaarden, A., and Jaenisch, R. (2012). Single-cell expression analyses during cellular reprogramming reveal an early stochastic and a late hierarchic phase. Cell *150*, 1209–1222.

Capel, B., Hawley, R.G., and Mintz, B. (1990). Long- and short-lived murine hematopoietic stem cell clones individually identified with retroviral integration markers. Blood *75*, 2267–2270.

Claudinot, S., Nicolas, M., Oshima, H., Rochat, A., and Barrandon, Y. (2005). Long-term renewal of hair follicles from clonogenic multipotent stem cells. Proc. Natl. Acad. Sci. USA *102*, 14677–14682.

Cohen, A.R., Gomes, F.L.A.F., Roysam, B., and Cayouette, M. (2010). Computational prediction of neural progenitor cell fates. Nat. Methods *7*, 213–218.

Costa, M.R., Ortega, F., Brill, M.S., Beckervordersandforth, R., Petrone, C., Schroeder, T., Götz, M., and Berninger, B. (2011). Continuous live imaging of adult neural stem cell division and lineage progression in vitro. Development *138*, 1057–1068.

Coutu, D.L., and Schroeder, T.T. (2013). Probing cellular processes by long-term live imaging—historic problems and current solutions. J. Cell Sci. *126*, 3805–3815.

de Souza, N. (2014). Screening CRISPly in human cells. Nat. Methods *11*, 125.

Deng, Q., Ramsköld, D., Reinius, B., and Sandberg, R. (2014). Single-cell RNA-seq reveals dynamic, random monoallelic gene expression in mammalian cells. Science *343*, 193–196.

Du, Z., Santella, A., He, F., Tiongson, M., and Bao, Z. (2014). De novo inference of systems-level mechanistic models of development from live-imaging-based phenotype analysis. Cell *156*, 359–372.

Eilken, H.M., Nishikawa, S.-I., and Schroeder, T. (2009). Continuous single-cell imaging of blood generation from haemogenic endothelium. Nature *457*, 896–900.

Eilken, H., Rieger, M., Hoppe, P., Hermann, A., Smejkal, B., Thum, M., Ninkovic, J., Beckervordersandforth, R., and Schoeder, T. (2011). Continuous long-term detection of live cell surface markers by "in culture" antibody staining. Protoc. Exch. Published online January 28, 2011. http://dx.doi.org/10.1038/protex.2011.205.

Endele, M., Etzrodt, M., and Schroeder, T. (2014). Instruction of hematopoietic lineage choice by cytokine signaling. Exp. Cell Res., in press. Published online July 18, 2014. http://dx.doi.org/10.1016/j.yexcr.2014.07.011.

Enver, T., Heyworth, C.M., and Dexter, T.M. (1998). Do stem cells play dice? Blood *92*, 348–351, discussion 352.

Faddah, D.A., Wang, H., Cheng, A.W., Katz, Y., Buganim, Y., and Jaenisch, R. (2013). Single-cell analysis reveals that expression of nanog is biallelic and equally variable as that of other pluripotency factors in mouse ESCs. Cell Stem Cell *13*, 23–29.

Faust, N., Varas, F., Kelly, L.M., Heck, S., and Graf, T. (2000). Insertion of enhanced green fluorescent protein into the lysozyme gene creates mice with green fluorescent granulocytes and macrophages. Blood *96*, 719–726.

Filipczyk, A., Gkatzis, K., Fu, J., Hoppe, P.S., Lickert, H., Anastassiadis, K., and Schroeder, T. (2013). Biallelic expression of nanog protein in mouse embryonic stem cells. Cell Stem Cell *13*, 12–13.

Frey, O., Misun, P.M., Fluri, D.A., Hengstler, J.G., and Hierlemann, A. (2014). Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis. Nat. Commun. *5*, 4250.

Frumkin, D., Wasserstrom, A., Itzkovitz, S., Stern, T., Harmelin, A., Eilam, R., Rechavi, G., and Shapiro, E. (2008). Cell lineage analysis of a mouse tumor. Cancer Res. *68*, 5924–5931.

Gazit, R., Mandal, P.K., Ebina, W., Ben-Zvi, A., Nombela-Arrieta, C., Silberstein, L.E., and Rossi, D.J. (2014). Fgd5 identifies hematopoietic stem cells in the murine bone marrow. J. Exp. Med. *211*, 1315–1331.

Germain, R.N. (2014). Open questions: a rose is a rose is a rose—or not? BMC Biol. *12*, 2.

Gerrits, A., Dykstra, B., Kalmykowa, O.J., Klauke, K., Verovskaya, E., Broekhuis, M.J.C., de Haan, G., and Bystrykh, L.V. (2010). Cellular barcoding tool for clonal analysis in the hematopoietic system. Blood *115*, 2610–2618.

Giesen, C., Wang, H.A., Schapiro, D., Zivanovic, N., Jacobs, A., Hattendorf, B., Schüffler, P.J., Grolimund, D., Buhmann, J.M., Brandt, S., et al. (2014). Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. Nat. Methods *11*, 417–422.

Gomez, D., Shankman, L.S., Nguyen, A.T., and Owens, G.K. (2013). Detection of histone modifications at specific gene loci in single cells in histological sections. Nat. Methods *10*, 171–177.

Gómez-Sjöberg, R., Leyrat, A.A., Pirone, D.M., Chen, C.S., and Quake, S.R. (2007). Versatile, fully automated, microfluidic cell culture system. Anal. Chem. *79*, 8557–8563.

Grégori, G., Rajwa, B., Patsekin, V., Jones, J., Furuki, M., Yamamoto, M., and Paul Robinson, J. (2014). Hyperspectral cytometry. Curr. Top. Microbiol. Immunol. *377*, 191–210.

Guo, G., Huss, M., Tong, G.Q., Wang, C., Li Sun, L., Clarke, N.D., and Robson, P. (2010). Resolution of cell fate decisions revealed by single-cell gene expression analysis from zygote to blastocyst. Dev. Cell *18*, 675–685.

Guo, M.T., Rotem, A., Heyman, J.A., and Weitz, D.A. (2012). Droplet microfluidics for high-throughput biological assays. Lab Chip *12*, 2146–2155.

Guo, G., Luc, S., Marco, E., Lin, T.-W., Peng, C., Kerenyi, M.A., Beyaz, S., Kim, W., Xu, J., Das, P.P., et al. (2013a). Mapping cellular hierarchy by single-cell analysis of the cell surface repertoire. Cell Stem Cell *13*, 492–505.

Guo, H., Zhu, P., Wu, X., Li, X., Wen, L., and Tang, F. (2013b). Single-cell methylome landscapes of mouse embryonic stem cells and early embryos analyzed using reduced representation bisulfite sequencing. Genome Res. *23*, 2126–2135.

Höckendorf, B., Thumberger, T., and Wittbrodt, J. (2012). Quantitative analysis of embryogenesis: a perspective for light sheet microscopy. Dev. Cell *23*, 1111–1120.

Holt, N., Wang, J., Kim, K., Friedman, G., Wang, X., Taupin, V., Crooks, G.M., Kohn, D.B., Gregory, P.D., Holmes, M.C., and Cannon, P.M. (2010). Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nat. Biotechnol. *28*, 839–847.

Hou, Y., Fan, W., Yan, L., Li, R., Lian, Y., Huang, J., Li, J., Xu, L., Tang, F., Xie, X.S., and Qiao, J. (2013). Genome analyses of single human oocytes. Cell *155*, 1492–1506.

Hughes, A.J., Spelke, D.P., Xu, Z., Kang, C.-C., Schaffer, D.V., and Herr, A.E. (2014). Single-cell western blotting. Nat. Methods *11*, 749–755.

Jaitin, D.A., Kenigsberg, E., Keren-Shaul, H., Elefant, N., Paul, F., Zaretsky, I., Mildner, A., Cohen, N., Jung, S., Tanay, A., and Amit, I. (2014). Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. Science *343*, 776–779.

Jordan, C.T., and Lemischka, I.R. (1990). Clonal and systemic analysis of long-term hematopoiesis in the mouse. Genes Dev. *4*, 220–232.

Junker, J.P., and van Oudenaarden, A. (2014). Every cell is special: genome-wide studies add a new dimension to single-cell biology. Cell *157*, 8–11.

Keller, P.J., Schmidt, A.D., Wittbrodt, J., and Stelzer, E.H.K. (2008). Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. Science *322*, 1065–1069.

Kemper, K., Prasetyanti, P.R., De Lau, W., Rodermond, H., Clevers, H., and Medema, J.P. (2012). Monoclonal antibodies against Lgr5 identify human colorectal cancer stem cells. Stem Cells *30*, 2378–2386.

Kirkegaard, K., Kesmodel, U.S., Hindkjær, J.J., and Ingerslev, H.J. (2013). Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study. Hum. Reprod. *28*, 2643–2651.

Kissa, K., and Herbomel, P. (2010). Blood stem cells emerge from aortic endothelium by a novel type of cell transition. Nature *464*, 112–115.

Klein, A.M., Nakagawa, T., Ichikawa, R., Yoshida, S., and Simons, B.D. (2010). Mouse germ line stem cells undergo rapid and stochastic turnover. Cell Stem Cell *7*, 214–224.

Kretzschmar, K., and Watt, F.M. (2012). Lineage tracing. Cell *148*, 33–45.

Kueh, H.Y., Champhekar, A., Nutt, S.L., Elowitz, M.B., and Rothenberg, E.V. (2013). Positive feedback between PU.1 and the cell cycle controls myeloid differentiation. Science *341*, 670–673.

Landry, Z.C., Giovanonni, S.J., Quake, S.R., and Blainey, P.C. (2013). Optofluidic cell selection from complex microbial communities for single-genome analysis. Methods Enzymol. *531*, 61–90.

Lecault, V., Vaninsberghe, M., Sekulovic, S., Knapp, D.J.H.F., Wohrer, S., Bowden, W., Viel, F., McLaughlin, T., Jarandehei, A., Miller, M., et al. (2011). High-throughput analysis of single hematopoietic stem cell proliferation in microfluidic cell culture arrays. Nat. Methods *8*, 581–586.

Lee, J.H., Daugharthy, E.R., Scheiman, J., Kalhor, R., Yang, J.L., Ferrante, T.C., Terry, R., Jeanty, S.S.F., Li, C., Amamoto, R., et al. (2014). Highly multiplexed subcellular RNA sequencing in situ. Science *343*, 1360–1363.

Li, H.H., Gyllensten, U.B., Cui, X.F., Saiki, R.K., Erlich, H.A., and Arnheim, N. (1988). Amplification and analysis of DNA sequences in single human sperm and diploid cells. Nature *335*, 414–417.

Lionnet, T., Czaplinski, K., Darzacq, X., Shav-Tal, Y., Wells, A.L., Chao, J.A., Park, H.Y., de Turris, V., Lopez-Jones, M., and Singer, R.H. (2011). A transgenic mouse for in vivo detection of endogenous labeled mRNA. Nat. Methods *8*, 165–170.

Liu, X., Long, F., Peng, H., Aerni, S.J., Jiang, M., Sánchez-Blanco, A., Murray, J.I., Preston, E., Mericle, B., Batzoglou, S., et al. (2009). Analysis of cell fate from single-cell gene expression profiles in C. elegans. Cell *139*, 623–633.

Livet, J., Weissman, T.A., Kang, H., Draft, R.W., Lu, J., Bennis, R.A., Sanes, J.R., and Lichtman, J.W. (2007). Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. Nature *450*, 56–62.

Loulier, K., Barry, R., Mahou, P., Le Franc, Y., Supatto, W., Matho, K.S., Ieng, S., Fouquet, S., Dupin, E., Benosman, R., et al. (2014). Multiplex cell and lineage tracking with combinatorial labels. Neuron *81*, 505–520.

Lovatt, D., Ruble, B.K., Lee, J., Dueck, H., Kim, T.K., Fisher, S., Francis, C., Spaethling, J.M., Wolf, J.A., Grady, M.S., et al. (2014). Transcriptome in vivo analysis (TIVA) of spatially defined single cells in live tissue. Nat. Methods *11*, 190–196.

Lu, R., Neff, N.F., Quake, S.R., and Weissman, I.L. (2011). Tracking single hematopoietic stem cells in vivo using high-throughput sequencing in conjunction with viral genetic barcoding. Nat. Biotechnol. *29*, 928–933.

Lu, S., Zong, C., Fan, W., Yang, M., Li, J., Chapman, A.R., Zhu, P., Hu, X., Xu, L., Yan, L., et al. (2012). Probing meiotic recombination and aneuploidy of single sperm cells by whole-genome sequencing. Science *338*, 1627–1630.

Lubeck, E., and Cai, L. (2012). Single-cell systems biology by super-resolution imaging and combinatorial labeling. Nat. Methods *9*, 743–748.

Ludin, A., Itkin, T., Gur-Cohen, S., Mildner, A., Shezen, E., Golan, K., Kollet, O., Kalinkovich, A., Porat, Z., D'Uva, G., et al. (2012). Monocytes-macrophages that express α-smooth muscle actin preserve primitive hematopoietic cells in the bone marrow. Nat. Immunol. *13*, 1072–1082.

Lutolf, M.P., Gilbert, P.M., and Blau, H.M. (2009). Designing materials to direct stem-cell fate. Nature *462*, 433–441.

MacArthur, B.D., Sevilla, A., Lenz, M., Müller, F.-J., Schuldt, B.M., Schuppert, A.A., Ridden, S.J., Stumpf, P.S., Fidalgo, M., Ma'ayan, A., et al. (2012). Nanog-dependent feedback loops regulate murine embryonic stem cell heterogeneity. Nat. Cell Biol. *14*, 1139–1147.

Mascré, G., Dekoninck, S., Drogat, B., Youssef, K.K., Broheé, S., Sotiropoulou, P.A., Simons, B.D., and Blanpain, C. (2012). Distinct contribution of stem and progenitor cells to epidermal maintenance. Nature *489*, 257–262.

Massberg, S., Schaerli, P., Knezevic-Maramica, I., Köllnberger, M., Tubo, N., Moseman, E.A., Huff, I.V., Junt, T., Wagers, A.J., Mazo, I.B., and von Andrian, U.H. (2007). Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. Cell *131*, 994–1008.

Mazutis, L., Gilbert, J., Ung, W.L., Weitz, D.A., Griffiths, A.D., and Heyman, J.A. (2013). Single-cell analysis and sorting using droplet-based microfluidics. Nat. Protoc. *8*, 870–891.

Micheva, K.D., and Smith, S.J. (2007). Array tomography: a new tool for imaging the molecular architecture and ultrastructure of neural circuits. Neuron *55*, 25–36.

Moignard, V., Macaulay, I.C., Swiers, G., Buettner, F., Schütte, J., Calero-Nieto, F.J., Kinston, S., Joshi, A., Hannah, R., Theis, F.J., et al. (2013). Characterization of transcriptional networks in blood stem and progenitor cells using high-throughput single-cell gene expression analysis. Nat. Cell Biol. *15*, 363–372.

Nagano, T., Lubling, Y., Stevens, T.J., Schoenfelder, S., Yaffe, E., Dean, W., Laue, E.D., Tanay, A., and Fraser, P. (2013). Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature *502*, 59–64.

Naik, S.H., Perié, L., Swart, E., Gerlach, C., van Rooij, N., de Boer, R.J., and Schumacher, T.N. (2013). Diverse and heritable lineage imprinting of early haematopoietic progenitors. Nature *496*, 229–232.

Naik, S.H., Schumacher, T.N., and Perié, L. (2014). Cellular barcoding: a technical appraisal. Exp. Hematol. *42*, 598–608.

Nakagawa, T., Sharma, M., Nabeshima, Y., Braun, R.E., and Yoshida, S. (2010). Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. Science *328*, 62–67.

Navin, N., Kendall, J., Troge, J., Andrews, P., Rodgers, L., McIndoo, J., Cook, K., Stepansky, A., Levy, D., Esposito, D., et al. (2011). Tumour evolution inferred by single-cell sequencing. Nature *472*, 90–94.

Newman, R.H., Fosbrink, M.D., and Zhang, J. (2011). Genetically encodable fluorescent biosensors for tracking signaling dynamics in living cells. Chem. Rev. *111*, 3614–3666.

Nguyen, P.K., Riegler, J., and Wu, J.C. (2014). Stem cell imaging: from bench to bedside. Cell Stem Cell *14*, 431–444.

Okita, C., Sato, M., and Schroeder, T. (2004). Generation of optimized yellow and red fluorescent proteins with distinct subcellular localization. Biotechniques *36*, 418–422, 424.

Osawa, M., Hanada, K., Hamada, H., and Nakauchi, H. (1996). Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science *273*, 242–245.

Ozawa, T., Natori, Y., Sato, M., and Umezawa, Y. (2007). Imaging dynamics of endogenous mitochondrial RNA in single living cells. Nat. Methods *4*, 413–419.

Park, D., Spencer, J.A., Koh, B.I., Kobayashi, T., Fujisaki, J., Clemens, T.L., Lin, C.P., Kronenberg, H.M., and Scadden, D.T. (2012). Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. Cell Stem Cell *10*, 259–272.

Pittet, M.J., and Weissleder, R. (2011). Intravital imaging. Cell *147*, 983–991.

Purvis, J.E., and Lahav, G. (2013). Encoding and decoding cellular information through signaling dynamics. Cell *152*, 945–956.

Rieger, M.A., Hoppe, P.S., Smejkal, B.M., Eitelhuber, A.C., and Schroeder, T. (2009). Hematopoietic cytokines can instruct lineage choice. Science *325*, 217–218.

Rinkevich, Y., Mori, T., Sahoo, D., Xu, P.-X., Bermingham, J.R., Jr., and Weissman, I.L. (2012). Identification and prospective isolation of a mesothelial precursor lineage giving rise to smooth muscle cells and fibroblasts for mammalian internal organs, and their vasculature. Nat. Cell Biol. *14*, 1251–1260.

Ritsma, L., Ellenbroek, S.I.J., Zomer, A., Snippert, H.J., de Sauvage, F.J., Simons, B.D., Clevers, H., and van Rheenen, J. (2014). Intestinal crypt homeostasis revealed at single-stem-cell level by in vivo live imaging. Nature *507*, 362–365.

Rompolas, P., Deschene, E.R., Zito, G., Gonzalez, D.G., Saotome, I., Haberman, A.M., and Greco, V. (2012). Live imaging of stem cell and progeny behaviour in physiological hair-follicle regeneration. Nature *487*, 496–499.

Sacco, A., Doyonnas, R., Kraft, P., Vitorovic, S., and Blau, H.M. (2008). Self-renewal and expansion of single transplanted muscle stem cells. Nature *456*, 502–506.

Schroeder, T. (2008). Imaging stem-cell-driven regeneration in mammals. Nature *453*, 345–351.

Schroeder, T. (2011). Long-term single-cell imaging of mammalian stem cells. Nat. Methods *8* (*Suppl*), S30–S35.

Shackleton, M., Vaillant, F., Simpson, K.J., Stingl, J., Smyth, G.K., Asselin-Labat, M.-L., Wu, L., Lindeman, G.J., and Visvader, J.E. (2006). Generation of a functional mammary gland from a single stem cell. Nature *439*, 84–88.

Shalek, A.K., Satija, R., Adiconis, X., Gertner, R.S., Gaublomme, J.T., Raychowdhury, R., Schwartz, S., Yosef, N., Malboeuf, C., Lu, D., et al. (2013). Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature *498*, 236–240.

Shalek, A.K., Satija, R., Shuga, J., Trombetta, J.J., Gennert, D., Lu, D., Chen, P., Gertner, R.S., Gaublomme, J.T., Yosef, N., et al. (2014). Single-cell RNA-seq reveals dynamic paracrine control of cellular variation. Nature *510*, 363–369.

Shapiro, E., Biezuner, T., and Linnarsson, S. (2013). Single-cell sequencing-based technologies will revolutionize whole-organism science. Nat. Rev. Genet. *14*, 618–630.

Sieburg, H.B., Cho, R.H., Dykstra, B., Uchida, N., Eaves, C.J., and Muller-Sieburg, C.E. (2006). The hematopoietic stem compartment consists of a limited number of discrete stem cell subsets. Blood *107*, 2311–2316.

Smith, L.G., Weissman, I.L., and Heimfeld, S. (1991). Clonal analysis of hematopoietic stem-cell differentiation in vivo. Proc. Natl. Acad. Sci. USA *88*, 2788–2792.

Snippert, H.J., van der Flier, L.G., Sato, T., van Es, J.H., van den Born, M., Kroon-Veenboer, C., Barker, N., Klein, A.M., van Rheenen, J., Simons, B.D., and Clevers, H. (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell *143*, 134–144.

Spangrude, G.J., Heimfeld, S., and Weissman, I.L. (1988). Purification and characterization of mouse hematopoietic stem cells. Science *241*, 58–62.

Strack, R.L., Disney, M.D., and Jaffrey, S.R. (2013). A superfolding Spinach2 reveals the dynamic nature of trinucleotide repeat-containing RNA. Nat. Methods *10*, 1219–1224.

Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. *100*, 64–119.

Sun, J., Ramos, A., Chapman, B., Johnnidis, J.B., Le, L., Ho, Y.J., Klein, A., Hofmann, O., and Camargo, F.D. (2014). Clonal dynamics of native haematopoiesis. Nature *514*, 322–327.

Tang, F., Barbacioru, C., Bao, S., Lee, C., Nordman, E., Wang, X., Lao, K., and Surani, M.A. (2010). Tracing the derivation of embryonic stem cells from the inner cell mass by single-cell RNA-Seq analysis. Cell Stem Cell *6*, 468–478.

Tay, S., Hughey, J.J., Lee, T.K., Lipniacki, T., Quake, S.R., and Covert, M.W. (2010). Single-cell NF-kappaB dynamics reveal digital activation and analogue information processing. Nature *466*, 267–271.

Toettcher, J.E., Gong, D., Lim, W.A., and Weiner, O.D. (2011). Light-based feedback for controlling intracellular signaling dynamics. Nat. Methods *8*, 837–839.

Torisawa, Y.S., Spina, C.S., Mammoto, T., Mammoto, A., Weaver, J.C., Tat, T., Collins, J.J., and Ingber, D.E. (2014). Bone marrow-on-a-chip replicates hematopoietic niche physiology in vitro. Nat. Methods *11*, 663–669.

Tumbar, T., Guasch, G., Greco, V., Blanpain, C., Lowry, W.E., Rendl, M., and Fuchs, E. (2004). Defining the epithelial stem cell niche in skin. Science *303*, 359–363.

Verovskaya, E., Broekhuis, M.J.C., Zwart, E., Ritsema, M., van Os, R., de Haan, G., and Bystrykh, L.V. (2013). Heterogeneity of young and aged murine hematopoietic stem cells revealed by quantitative clonal analysis using cellular barcoding. Blood *122*, 523–532.

Verovskaya, E., Broekhuis, M.J.C., Zwart, E., Weersing, E., Ritsema, M., Bosman, L.J., van Poele, T., de Haan, G., and Bystrykh, L.V. (2014). Asymmetry in skeletal distribution of mouse hematopoietic stem cell clones and their equilibration by mobilizing cytokines. J. Exp. Med. *211*, 487–497.

Wang, J., Fan, H.C., Behr, B., and Quake, S.R. (2012). Genome-wide single-cell analysis of recombination activity and de novo mutation rates in human sperm. Cell *150*, 402–412.

Warren, L., Bryder, D., Weissman, I.L., and Quake, S.R. (2006). Transcription factor profiling in individual hematopoietic progenitors by digital RT-PCR. Proc. Natl. Acad. Sci. USA *103*, 17807–17812.

Weibrecht, I., Leuchowius, K.-J., Clausson, C.-M., Conze, T., Jarvius, M., Howell, W.M., Kamali-Moghaddam, M., and Söderberg, O. (2010). Proximity ligation assays: a recent addition to the proteomics toolbox. Expert Rev. Proteomics *7*, 401–409.

Wells, D. (2014). Next-generation sequencing: the dawn of a new era for preimplantation genetic diagnostics. Fertil. Steril. *101*, 1250–1251.

White, A.K., VanInsberghe, M., Petriv, O.I., Hamidi, M., Sikorski, D., Marra, M.A., Piret, J., Aparicio, S., and Hansen, C.L. (2011). High-throughput microfluidic single-cell RT-qPCR. Proc. Natl. Acad. Sci. USA *108*, 13999–14004.

Whitlock, C.A., and Witte, O.N. (1982). Long-term culture of B lymphocytes and their precursors from murine bone marrow. Proc. Natl. Acad. Sci. USA *79*, 3608–3612.

Whitlock, C.A., Tidmarsh, G.F., Muller-sieburg, C., and Weissman, I.L. (1987). Bone Marrow Stromal Cell Lines with Lymphopoietic Activity Express High Levels of a Pre-B Molecule. Cell *48*, 1009–1021.

Willison, K.R., and Klug, D.R. (2013). Quantitative single cell and single molecule proteomics for clinical studies. Curr. Opin. Biotechnol. *24*, 745–751.

Wright, D.E., Wagers, A.J., Gulati, A.P., Johnson, F.L., and Weissman, I.L. (2001). Physiological migration of hematopoietic stem and progenitor cells. Science *294*, 1933–1936.

Xu, X., Hou, Y., Yin, X., Bao, L., Tang, A., Song, L., Li, F., Tsang, S., Wu, K., Wu, H., et al. (2012). Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor. Cell *148*, 886–895.

Yamamoto, R., Morita, Y., Ooehara, J., Hamanaka, S., Onodera, M., Rudolph, K.L., Ema, H., and Nakauchi, H. (2013). Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. Cell *154*, 1112–1126.

Yan, L., Yang, M., Guo, H., Yang, L., Wu, J., Li, R., Liu, P., Lian, Y., Zheng, X., Yan, J., et al. (2013). Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. Nat. Struct. Mol. Biol. *20*, 1131–1139.

Youssef, K.K., Van Keymeulen, A., Lapouge, G., Beck, B., Michaux, C., Achouri, Y., Sotiropoulou, P.A., and Blanpain, C. (2010). Identification of the cell lineage at the origin of basal cell carcinoma. Nat. Cell Biol. *12*, 299–305.

Zhao, J.L., Ma, C., O'Connell, R.M., Mehta, A., DiLoreto, R., Heath, J.R., and Baltimore, D. (2014). Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis. Cell Stem Cell *14*, 445–459.

Zong, C., Lu, S., Chapman, A.R., and Xie, X.S. (2012). Genome-wide detection of single-nucleotide and copy-number variations of a single human cell. Science *338*, 1622–1626.