

# The living–tissue microscope: the importance of studying stem cells in their natural, undisturbed microenvironment<sup>#</sup>

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## Abstract

Advances in stem cell research highlight the importance of analysing multicellular interactions *in vivo* before modelling them in cell culture systems. Gain-of-function assays such as transplantation are useful, but are not equivalent to studying cells in their natural, undisturbed microenvironment.

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**Keywords:** stem cell; clonal unit; *in situ* assay; microenvironment; lineage analysis; live cell imaging

<sup>#</sup> Commentary in relation to: Gaisa NT, Graham TA, McDonald SAC, et al. The human urothelium consists of multiple clonal units each maintained by a stem cell. J Pathol 2011; 225: 163–171. DOI: 10.1002/path.2945 and also in relation to Blackwood JK, Williamson SC, Greaves LC, et al. *In situ* lineage tracking of human prostatic epithelial stem cell fate reveals a common clonal origin for basal and luminal cells. J Pathol 2011; 225: 181–188. DOI 10.1002/path.2965. and Gaisa NT, Graham TA, McDonald SAC, et al. Clonal architecture of human prostatic epithelium in benign and malignant conditions. J Pathol 2011; 225: 172–180. DOI 10.1002/path.2959.

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Both science and art have always been limited by tools; there were no Michelangelos before the chisel. Today's biological Renaissance requires an equally fundamental instrument: one that reports the structure, location, movement and genotype of every tissue cell, while the cells go about their business, unperturbed. Peering through such a 'living-tissue microscope' would help clarify virtually every aspect of normal physiology and disease, and would reveal novel cellular and genetic pathways throughout the body unsuspected from fixed material. Fortunately, we live in an era where many of these capabilities are emerging. Small multicellular structures expressing a handful of tagged genes can now be imaged live at single-cell resolution for many hours [1–3]. Furthermore, advances in genetics enable researchers to use lineage analysis to record the growth and movement of individual tissue cells over extended time periods. In this issue, Gaisa *et al* [4] utilize spontaneous mitochondrial DNA mutations arising within individual cells to carry out lineage analysis within the human bladder. Their observations provide novel insight into the location and behaviour of uroepithelial stem cells. The approach will have widespread applicability.

Stem cell research particularly requires the ability to follow cellular behaviour *in vivo*, because tissue stem cells are rare and almost impossible to initially identify by appearance or marker gene expression (reviewed in [5–7]). However, lineage analysis can unambiguously reveal stem cells by fingering rare cells that self-renew. Decades ago, when systematically following cell behaviour *in vivo* was virtually

impossible, the field made great advances by measuring functional stem cells. In a few favourable tissues, such as bone marrow and testis, a tiny subpopulation of dissociated tissue cells was shown to reconstitute cell production following transplantation into hosts with compromised stem cell activity. These studies allowed stem cells to be enriched and documented stem cell niches, specific tissue subregions needed to maintain added stem cells. However, it was impossible to determine whether all the rescuing cells normally function as stem cells. Eventually, studies in *Drosophila* and mice proved that many differentiating early lineage cells are capable of becoming stem cells if they gain niche access (reviewed in [5–7]). Thus, tissues often contain many more potential than actual stem cells, because actual stem cell number is limited by niche availability. While transplantation can measure the former, monitoring cell behaviour *in vivo* is necessary to pinpoint actual stem cells in their natural state.

Currently, stem cells have not been precisely localized within most mammalian tissues. Lineage-based methods need to be applied throughout all the organs of the body to rectify this situation. Until very recently stem cell searches routinely employed assays based on dubious assumptions about stem cell behaviour. For example, most stem cells were thought to be quiescent and to cycle only sporadically. This led some researchers to equate cells that remain labelled long after a BrdU pulse with stem cells. However, the activity of at least six types of stem cells have now been characterized at single-cell resolution by *in vivo* lineage studies in *Drosophila* and mice (reviewed in

[5–7]). All were observed to divide continuously, although at rates modulated by environmental factors. Clearly, label retention has not proved to be a reliable stem cell identifier.

Stem cells have also been assumed to spawn large and/or diverse colonies following explantation into tissue culture. The logic underlying this expectation is flawed, however. In most cases, ‘stemness’ does not appear to be an autonomous cellular property that persists in culture, but a labile state maintained by the tissue microenvironment. Stem cells *in vivo* generally differentiate if they leave or are displaced from the niche (reviewed in [5–7]). Even haematopoietic stem cells, whose exceptional stability may facilitate movement around the body, are difficult to expand *in vitro* [8,9], a situation that may not change until their niches are better characterized and can be simulated. Successful propagation of *Drosophila* germline stem cells [10] or mouse intestinal stem cells [11,12] requires specific cytokines found in the niche, as well as actual niche cells, which may provide extracellular matrix and surface factors. Effective culture conditions were discovered only after these stem cells were identified by lineage analysis and normal niche cells and signals were characterized. Without a physiological microenvironment, colony growth in culture may bear no relationship to cellular behaviour *in vivo*.

What is true of stem cells and niches likely applies to many other aspects of tissue development and physiology. Embryonic cells signal continuously during development and mature tissue cells continue to interact with their neighbours, with their immediate environment and with general humoral signals. When dissociated, tissue cells lose the microenvironment that supports their normal activity, much as stem cells do when removed from the niche. Cells in culture frequently undergo genetic and epigenetic changes, regardless of whether they retain a grossly normal karyotype [13–15]. These genomic alterations likely result from the non-physiological signals and stresses cells experience when their normal microenvironment is not accurately replicated. These devastating effects highlight the advantages of culturing tissues rather than cells, and indicate that today’s cell cultures are better indicators of cellular rather than multicellular biology.

Stem cell research has taught us that if cell cultures are to be successfully employed to probe non-autonomous processes, a new approach is called for. The processes of interest must be studied first within intact tissues *in vivo* or in culture. When enough has been learned about the cells involved and their microenvironments, a sophisticated cell culture scheme can be designed. Studies at the single cell level must then be carried out to verify that the cells *in vitro* are behaving normally. Only by following this approach can one reasonably expect to replicate the intricate intercellular networks on which metazoan development and physiology depend. Ongoing improvements in live imaging and lineage analysis, including those employed in recent papers in this journal [4,16,17],

will increasingly make this powerful route available, allowing investigators to gain the experimental advantages that a cell culture system can provide, without destroying the biological processes that they seek to understand.

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[Correction added after online publication 5 August 2011: a typographical error in the Abstract and References section was corrected.]



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Original Paper

## Clonal architecture of human prostatic epithelium in benign and malignant conditions†

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**Keywords:** prostate; stem cells; clonality; PIN; prostate cancer

### Abstract

The location of stem cells in the epithelium of the prostatic acinus remains uncertain, as does the cellular origin of prostatic neoplasia. Here, we apply lineage tracing to visualize the clonal progeny of stem cells in benign and malignant human prostates and understand the clonal architecture of this epithelium. Cells deficient for the mitochondrially-encoded enzyme cytochrome c oxidase (CCO) were identified in 27 frozen prostatectomy specimens using dual colour enzyme histochemistry and individual CCO-normal and -deficient cell areas were laser-capture microdissected. PCR-sequencing of the entire mitochondrial genome (mtDNA) of cells from CCO-deficient areas found to share mtDNA mutations not present in adjacent CCO-normal cells, thus proving a clonal origin. Immunohistochemistry was performed to visualize the three cell lineages normally present in the prostatic epithelium. Entire CCO-deficient acini, and part-deficient acini were found. Deficient patches spanned either basal or luminal cells, but sometimes also both epithelial cell types in normal, hyperplastic or atrophic epithelium, and prostatic intraepithelial neoplasia (PIN). Patches comprising both PIN and invasive cancer were observed. Each cell area within a CCO-deficient patch contained an identical mtDNA mutation, defining the patch as a clonal unit. CCO-deficient patches in benign epithelium contained basal, luminal and endocrine cells, demonstrating multilineage differentiation and therefore the presence of a stem cell. Our results demonstrate that the normal, atrophic, hypertrophic and atypical (PIN) epithelium of human prostate contains stem cell-derived clonal units that actively replenish the epithelium during ageing. These deficient areas usually included the basal compartment indicating the basal layer as the location of the stem cell. Importantly, single clonal units comprised both PIN and invasive cancer, supporting PIN as the pre-invasive lesion for prostate cancer. Copyright © 2011 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

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