

## (Re)Defining tissues: A review and revision of histology's nomenclature

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### ABSTRACT

Histology's nomenclature is revised in order to incorporate the tissue dynamics of stem cells. Commonly, tissues are sorted into two static classes on the basis of the relationship of cells to extracellular material: An epithelial-like class consists of epithelia, muscle, nerve, and germ, and an ameba-like class consists of vascular and connective tissue. Dynamically, epithelia are either proto-epithelia comprising parenchymal cache cells (CCs) or meta-epithelia with parenchymal self-renewing adult stem cells (ASCs), clones of transitional amplifying cells (TACs), and terminally differentiating cells (TDCs). Muscle is either cellular (cardiac and smooth) exhibiting CC-like dynamics or syncytial (skeletal muscle) with differentiated fibers and undifferentiated reserve cells (RCs, aka satellite cells). Nerve contains adult stem-like (AS-like) cells that give rise to terminally differentiated (TD-like) neuroblasts and glioblasts. Female germ tissue in mammals probably contains AS-like cells or RCs and their progeny, while male germ tissue contains both AS-like cells and RCs and their progeny. Vascular tissue contains multipotent AS-like hematopoietic stem cells and their progeny. Connective tissue (CT) consists of fixed and mobile CC-like cells, the latter possibly multipotent and available for recruitment. Adipose tissue has CC-like qualities. Conceivably, tissue dynamics evolved through intra-organism competition of epithelia-like and ameba-like "ur-tissues".

**KEYWORDS:** adult stem cells (ASCs), cancer initiating cells (CICs), cancer stem cells (CSCs), cache cells (CCs), embryonic stem cells (ESCs), hematopoietic stem cells (HSCs), reserve cells (RCs), transit amplifying cells (TACs), terminally differentiated cells (TDCs)

### INTRODUCTION

Classical histology's descriptions of static tissues have been enormously successful for identifying and sorting tissues into coherent classes, explaining their coordinated activities in organs, and aiding in the diagnosis of disease. Contemporary histology would be well served were it to add dynamics to descriptions of tissues. Adult tissues are not after all generally static and taking their dynamic dimensions into account would be useful for understanding many roles played by cells in normal tissues and their pathological derivatives.

Dynamics have been most conspicuously useful in pathology and immunology. For example, by demonstrating "that the more isologous the relationship is between host and donor the less marrow material is necessary to provide protection or repopulation" [1], and that better integration in biorhythm provides better bio-effectiveness of chemotherapeutic drugs [2] and more "predictable changes in tolerability and ... long-term survival" [3].

Regrettably, "after 30 years of debate" [4], dynamics have not been incorporated into histology's nomenclature. Histologists do not distinguish

between tissues having adult (organ or tissue) stem cells (ASCs) as opposed to tissues lacking these cells [5]; indicate how stem cells originate, whether through the differentiation of organ primordia or via multipotential embryonic stem cells (ESCs); how ASCs are integrated into tissue dynamics; whether transit amplifying cells (TACs or precursors) are multi-potential, oligo-potential, or mono-potential; how TACs are distributed and their fate(s) regulated; how stem cell distribution changes with age; whether cancer stem cells (CSCs, aka cancer initiating cells [CICs]) arise from ASCs [6], TACs, and/or are induced from less aggressive cancer cells [7]. Indeed, vacillation around definitions and usage of the term “stem cell” has created ambiguity in tissue dynamics [8].

While contemporary cell biology is burgeoning with reports on dynamics [e.g., 9, 10], literally thousands of cell types go unrecognized by histologists despite vastly different morphological, physiological, and histochemical behavior [11]. Similar obstacles confront research on interactions within a tissue [12] and between tissues [13]. The remedy, however, is at hand: updating histology’s nomenclature to incorporate dynamics into histology’s traditional descriptive classes of tissues. Histology’s potential is enormous, and introducing dynamical thinking and practice into histology’s language will allow that potential to blossom.

### **Histology’s nomenclature**

Histology’s successes in the past have not been achieved without confronting difficulty [14, 15, 16]. Since the mid-19<sup>th</sup> century, histologists have diligently met the challenge of translating the two-dimensional microscopic field of sections into the three-dimensional macroscopic space of organs and converting static microscopic images to descriptions of living tissues [17]. Today, histology’s nomenclature must respond to contemporary problems by incorporating the vocabulary and usage of dynamics revealed through modern microscopy.

Today, biomarkers, immunofluorescence, confocal microscopy, and fluorescent flow cytometry hold promise for providing a precise and systematic language for characterizing stem cells in tissue and plotting the course of tissue dynamics [18].

The goal is to reach consensus on a list of stem cell antigens [19] or markers as “molecular signatures” for stem cells [20, 21].

Although some tissues may grow by addition via the recruitment of proliferative foreign cells, four types of proliferative cells are inherent members of different adult tissues: adult stem cells (ASCs), transit amplifying cells (TACs), cache cells (CCs [as in carbon copies]), and reserve cells (RCs).

ASCs are also known as actual and somatic stem cells. They constitute a small part of a steady-state adult tissues’ proliferative population that exhibits self-renewal (or asymmetric division) dividing at a slow rate and giving rise to clones of TACs.

TACs are also known as transitional amplifying cells, progenitors or precursors. According to Ronald McKay “The term ‘progenitor’ refers to a cell with a more restricted potential than a stem cell. ‘Precursor’ is a less stringent term that refers to any cell that is earlier in a developmental pathway than another” [22]. TACs divide rapidly and give rise to non-dividing terminally differentiating cells (TDCs).

CCs are differentiated cells that reserve the ability to divide. Dividing CCs produce additional CCs.

RCs are undifferentiated cells that have suspended division [23, 24]. RCs resemble ASCs when mobilized to divide (possibly only briefly) by trauma or stress.

Inevitably there is some overlap. For example, liver maintenance may normally be performed by hepatic CCs, whereas a liver with its regenerative capacity exhausted by severe or chronic liver disease may yet regenerate as a function of small, oval adult stem-like cells (AS-like cells) originating from cholangioles (aka canals of Hering). Similarly, mobilizing foreign cells locally or recruiting them from afar may occur through combinations of local and systemic stimuli and coordination. For example, liver regeneration may be enhanced by extrahepatic mesenchymal stem cells recruited from bone marrow [25].

As a small, slowly growing fraction of a larger proliferative population [26], ASCs are equated with label-retaining cells (LRCs) thought to collect the template or old DNA (i.e., not freshly

replicated strand of deoxynucleic acid) [27]. ASCs are also Hoechst 33342 and Rhodamine 123 dye excluding cells concentrated in the side population (SP) by fluorescent-activated cell sorting [28, 29]. Hopefully, ASCs will be identified by “cocktails of markers,” but the “gold standard” for ASCs is their morphological niche, a unique site populated exclusively by ASCs, sometimes *en masse* [30]. Ideally, anatomically distinctive niches, such as the bulge of the outer root sheath of hair follicles [31] have definable microanatomies (microenvironments) that concentrate ASCs, sequester or induce them, and nurture them specifically [32]. Creating coherence among different definitions or identifying different types of self-renewing stem cells remains a monumental challenge.

### Revised tissue nomenclature

In histology’s traditional nomenclature, tissues are placed into one of two superclasses: epithelial-like (epithelia, muscle, nerve, and germ) and ameba-like (vascular and connective tissue). Cells of epithelial-like tissues are mounted on or enclosed in membranous extracellular material (ECM such as basal lamina, external lamina, zona pellucida, and [possibly] decapacitation factor). The epithelial components of basal lamina include the glycoproteins entactin that binds laminin to type IV collagen. Similarly, zona pellucida proteins are produced by oocytes and by surrounding granulosa cells, and integrins (trans-membrane proteins with receptor sites for glycoproteins) on neuroblasts bind extracellular tenascin during nerve outgrowth in embryos. In addition, cells in the epithelial-like superclass tend to have complex intercellular connections (zonulae occludentes, zonulae adherents, maculae adherents, caveola, intercalated disks, nexus or gap junctions), more complex junctions (synaptic clefts), and even junctions with other tissues (myoneural junctions, stretch receptors, hemidesmosomes).

Cells of the ameba-like tissues are bathed or enclosed in copious three-dimensional extracellular material and largely lack intercellular connections and junctions. Vascular tissues’ cells may be sequestered in the interstices of connective tissue but while lacking conspicuous connections and junctions are remarkably adept at communicating

with each other (e.g., coupling signals leading to antibody production). Connective tissue, long identified with its ECM, contains fibers, notably collagen and elastin, “ground substance” of glycosaminoglycans, sulfated and unsulfated, and glycoproteins, fibrillin, fibronectin produced by fibroblasts or fibrocytes.

The cells of both epithelial-like and ameba-like tissues exhibit a range of proliferative patterns that suggest evolutionary series. Although the categories are not airtight and some overlap is conspicuous, in general, tissues with CC and CC-like proliferation probably represent ancestral types that gave rise to tissues with ASC and ASC-like proliferation. Likewise, RCs and RC-like proliferation patterns would seem derived from ASC tissues. Hence, epithelia and muscle with CC kinetics are called “proto-” and those with ASC and TAC kinetics are called “meta-”. Nerve and germ seem to be largely of the “meta-” variety. Similarly, connective tissue seems largely in the “proto-” class (evolving in parallel with epithelia’s CCs), while vascular tissue exhibits a hierarchical variation of the “meta-” variety (evolving convergent with epithelia’s ASC varieties).

### Epithelia

#### Proto-epithelia: General cell division

Proto-epithelia parenchymal cells are CCs, each of which has the capacity to divide but division may be more likely in some locales than others. CCs are differentiated (by morphological criteria) and their division contributes differentiated cells to the population. Cell division is not self-renewing and asymmetrical: Neither one cell nor the other produced by a division retains the preponderance of old (template) or new (replicated) DNA strands. Cell cycling is presumably controlled by cyclins, cyclin-dependent kinases, and may exhibit inherent circadian rhythms (e.g., in rat kidney [33], rat uterine luminal epithelium [34], mouse liver [35], and Ehrlich ascites carcinoma [36]).

CCs suffer from multiple points of vulnerability to malignant transformation. Curiously, cancer stem cells (CSCs) of glandular carcinomas may exhibit ASC-like self-renewal [37] suggesting a mismatch

between carcinoma cells and their original cell type. Malignancy may be the consequence of such a mismatch.

#### **Meta-epithelia: Self-renewing ASCs and clones of TACs**

Meta-epithelia contain two functionally different types of dividing parenchymal cells, ASCs and TACs, and one type of non-dividing parenchymal cell, TDCs differentiating from TACs. The population size of self-renewing ASCs (dividing asymmetrically) may not change with age [9, 38], although ASCs' competence may decline [39]. Signal transduction pathways originating in niches presumably influence ASC behavior [40], and ASCs also exhibit inherent circadian rhythms (e.g., in mouse cornea [41], hairless mouse dorsum [42], mouse tongue keratinocytes [43]). Remarkably, while this passage from ASC to TAC and TDC is fundamental to the normal turnover of cells in tissues [44], it may also be reversible [45], and RC may also be found in epithelia (e.g., pancreatic acini [46]).

ASCs may also be the source of CSCs [47, 48]. ASCs would seem to be vulnerable to malignant cancerous transformations via Wnt-pathway-activating mutations (e.g., *adenomatous polyposis coli* [*APC*]). In contrast, TACs seem to undergo malignant transformations no further than benign tumors [21]. Distinctions in the markers of ASCs and TACs, therefore, may be useful for distinguishing between malignant cancers and benign tumors (e.g., of the prostate and vagina).

#### **Muscle**

Muscle is epithelial-like in several ways. Indeed, muscle's motor protein, myosin resembles the nonmuscle myosin of epithelia. Remarkably, "vertebrate smooth muscle myosin is more similar to nonmuscle myosin than to striated muscle myosin, both in sequence and in biochemical characteristics ... [Amino acid sequences indicate that] smooth muscle and striated muscle myosins branch[ed] independently from nonmuscle myosin. ... [I]n fact, ... the two types of muscle tissue may also be independently derived from nonmuscle tissue" [49].

Other epithelial qualities present in muscle include the presence of an external lamina

resembling the basal lamina of epithelia; dense bodies in smooth muscle resembling desmosomes (anchoring points of intermediate filaments); nexus or gap junctions (between smooth muscle cells and between cardiomyocytes); fascia adherens (adhering junctions) in cardiac muscle resembling zona adherens in epithelia.

#### **Proto-muscle**

Proto-muscle or cellular muscle includes smooth and cardiac muscle. They exhibit cache-like dynamics (dividing differentiated cells).

The heart is a system of muscle, blood vessels, CT with its own intrinsic conductive system derived from muscle, all originating in multipotent stem cells: "the heart is built from a pool of multipotent cells that persists and differentiates as the heart grows" [50]. In mice and human beings, this cellular pool has multiple origins. Endothelial cells lining cardiac vasculature, vascular smooth muscle, and mesoderm form cardiovascular progenitor (aka colony forming) cells. In addition, embryonic proepicardial cells de-epithelialize (become mesenchymal), invade the developing heart, transiently express their T-box transcription factor (i.e., become *Tbx18*-expressing cardiac progenitors), and give rise to cardiomyocytes, interstitial cardiac fibroblasts, and coronary (vascular) smooth muscle cells [51]. Indeed, "a large proportion of proepicardial cells are pluripotent and can adopt either cardiomyocyte or smooth muscle cell fates" [52].

Furthermore, adult epicardial cells adopt angiogenic cell fates *in vitro* differentiating as fibroblasts, smooth muscle cells, and endothelium of vessels [53]. And aging myopathy in the heart is retarded in mice transgenic for insulin-like growth factor-1 (*IGF-1*) otherwise known to promote stem cell growth and survival [54].

In human beings specifically, fetal cardiac transcription factor *ISL1*<sup>+</sup> (islet 1 positive) containing cardiovascular progenitors display multipotency, giving rise to cardiomyocytes, smooth muscle, and endothelial cell lineages: In contrast, in "murine cardiogenesis, large numbers of multipotent *ISL1*<sup>+</sup> progenitors persist during late stages of human fetal cardiogenesis, suggesting a stem cell paradigm for the exponential growth of the human fetal heart and outflow tract over many weeks" [55].

The large numbers of cells expressing markers of cardiac tissue, cardiac myocytes, smooth muscle, and endothelium in adult tissue suggest that if cardiac regeneration following infarction occurs, it may be a function of cache-like cells [56]. Alternatively, if adult hearts are maintained and potentially restored by local cardiac stem-like cells (and possibly epicardial stem-like cells) a side population (SP) of heart cells should be demonstrated by fluorescent flow cytometry.

Alternatively, regeneration may occur through recruitment of cells from circulation [57]. Indeed, the migration of cells to sites of myocardial infarction is a tantalizing hypothesis for proponents of embryonic stem cell therapies. Myocardium formed following direct injection into peri-infarcted left ventricles of lineage-negative ( $\text{Lin}^-$ ) bone marrow cells obtained from transgenic mice by fluorescence-activated cell sorting for  $c\text{-kit}^{\text{POS}}$  stem cells [58]. But transplanted autologous skeletal myoblasts experience high rates of early cell death and limited success relieving infarcted myocardium [59].

The notion that differentiated smooth muscle cells resemble CCs of epithelia is traced back to an article by Earl Benditt on arteriosclerotic lesions containing variant (disorganized) smooth muscle (i.e., tunica media) cells in plaques [60]. Indeed, “Benditt’s observation of monoclonality also implied some intrinsic mechanism allowing cells to grow in a focal manner [61]. Benditt and others later concluded that, “smooth muscle cells from normal arteries can show monoclonal characteristics” [62].

On the other hand, label-retaining cells (LRCs) resembling ASCs are found in smooth muscle in the endometrium following pulse-chase experiments with labeled DNA analogues. A model system for the regeneration of mouse myometrium showed that after stimulation by human chorionic gonadotropin, LRCs produced clones of cells in the uterine stroma followed by growth in the myometrium. The “lineage differentiation of uterine myometrial cells may [therefore] represent a continuum, with LRCs being the most primitive, followed by ... transient amplifying cells, and finally terminal differentiation into myometrial cells” [63].

### Meta-muscle

Meta- (i.e. skeletal) muscle exhibits RC dynamics when satellite cells are provoked to divide. Sublaminal satellite cells are a distinguishing characteristic of mammalian skeletal muscle. Skeletal muscle is typically long-lived. Indeed, retrospective birth dating demonstrates that skeletal muscle of the rib cage turned over in fifteen to sixteen years [64]. But reserve or satellite cells may yet sustain growth and replacement. Division in satellite cells resembles that of ASCs by way of being self-renewing and asymmetric, returning the old DNA strand to the satellite cell and shunting the new DNA strand to a TA-like precursor [65] that divides and forms a (small) clone of myoblasts that, in turn, fuse and differentiate into fibers (myotubules). Indeed, while the typical satellite cell is  $\text{Pax7}^+/\text{Myf5}^-$ , its division contributes a  $\text{Pax7}^+/\text{Myf5}^-$  satellite cell to the reservoir of RCs and a  $\text{Pax7}^+/\text{Myf5}^+$  muscle precursor to the supply of differentiating skeletal myoblasts [66]. Uncertainty remains, however over the number of times satellite cells can divide and contribute to muscle regeneration [67].

Despite early reports to the contrary [68], injured striated muscles in adults seem incapable of successfully recruiting cells from remote sites. Indeed, the notion that skeletal muscle “can arise from non-satellite cells associated with blood vessels or interstitial tissue ... [is] challenged ... by the finding that virtually all satellite cells in regenerated muscle were marked as being derived from ... [satellite] progenitors” [69].

### Nerve

Some parts of the nervous system are not as static as once thought. For example, the subventricular zone (SVZ) “of the adult mammalian brain retains the potential to generate new neurons” [70]. Moreover, adult human neural progenitors (AHNPs) extracted from many areas of the adult forebrain and expanded in tissue culture subsequently differentiated into glial and neuronal cell types both *in vivo* and *in vitro* [71]. Similar stem-like cells are obtained from various parts of mammalian brains whether plated as monolayers in tissue culture or reared as floating aggregates called neurospheres [72]. Indeed, “in the past few years, scientists have found that certain kinds of neurons can grow in adult brains, including those

of humans. Not only has this turned scientific dogma on its head, but it has also provided the first glimmer of hope for those suffering from degenerative brain disease or paralyzing spinal cord injuries” [73].

The concept of shifting neuronal “germinal zones” containing AS-like neuronal stem cells (NSCs) has replaced the notion of a strictly static nervous system. The first germinal zone is the embryonic neuro-epithelium where mitotic activity takes place in the matrix near the outer surface producing neuroblasts and then glioblasts [74, 75]. The matrix is also called the ventricular zone (VZ), since it will later line the ventricle and neural canal formed when the neural plate folds into neural ridges that fuse dorsally into the neural tube. Subsequently, different mechanisms give rise to the cortical and non-cortical regions of the brain [76].

Mitotic activity moves peripherally into the second germinal zone, the subventricular zone (SVZ) where mitotic activity will remain in portions of the adult central nervous system (CNS). The cells that divide in the adult SVZ have the potential to differentiate into neurons and central glial cells [70], and some carry specific markers qualifying them as neural precursors. Specifically, some cells are positive for MAP-2 (microtubule-associated protein 2), NF (neurofilament), and NSE (neuron-specific enolase) but not for GFAP (glial fibrillary acidic protein). Proliferative SVZ cells also retain labeled DNA (i.e., they are LRCs), and exhibit self-renewal asymmetric cell division [77]. Furthermore, the post-natal SVZ germinal zone also qualifies as a niche: the extra-cellular matrix molecule tenascin C (highly expressed in the SVZ) promotes the accumulation and proliferation of NSCs with ectodermal growth factor receptors (EGFRs) [78].

Adult NSCs are especially reactive to growth factors. For example, intraventricular administration of mitogens such as epidermal growth factor (EGF) induces proliferation of subependymal cells, their migration away from lateral ventricle walls, and differentiation into astrocytes and neurons [79]. *In vitro*, combinations of EGF and basic fibroblast growth factor (bFGF) induce proliferation in cells obtained from the ependymal layer of mouse thoracic and lumbar/sacral

segments of the spinal cord and fourth ventricle of the brain [80].

Localized (circumscribed) germinal zones in the adult SVZ are found in the lateral ventricle of the brain, hippocampus [81, 22], and the subependymal regions of the spinal cord in mammals from mice to cows [82]. NSCs present in these regions produced TAC-like cells that differentiated preferentially as glia as opposed to neurons, but cells differentiating as neurons (i.e., neuroblasts) in rodents move chain-like in a rostral migratory stream (RMS) to the olfactory bulb where they become integrated granular interneurons [83].

Intriguingly, human neural precursor cells (hNPCs) obtained from fetal cortices and cultured *in vitro* formed neurospheres and grew rapidly in the presence of EGF and FGF-2. The number of cells expressing the neuron specific marker,  *$\beta$ -tubulin III* increased when the neurospheres were allowed to settle on coverslips, and hNPCs differentiated into neuron-like cells [84]. Similar changes were found in precursors derived from the neural crest in the enteric nervous system [85].

### Germ tissue

Estimates of the number of oocytes at birth suggest that mammalian ovaries simply do not contain enough oocytes enclosed within primitive follicles to support all the oocytes released at ovulation and the far greater number that die in atretic (unperforated) follicles during the female’s reproductive lifetime [86]. Problems with estimates of early follicle number leave room for doubt [87], however. One possible source of additional eggs would be the mesothelium surrounding the ovary, or ovarian surface epithelium (OSE) [88], once called the germinal epithelium. Renewal of oocytes from the OSE has been demonstrated experimentally [89], and markers indicate that the production of new oocytes is not due to the recruitment of circulating germ cells [90].

Thus, adult female mammals, like their male counterpart may have germinal stem cells (GSCs) lying in wait as reserve germinal cells (RGCs). Possibly, cryptic oogonia in the form of GSCs or RGCs passed through an establishment phase during development and came to reside in a maintenance (reserve) or proliferative (self-renewing) phase in adults. The establishment

phase would be governed by extracellular signals from hormones and by local interactions, while the maintenance phase would be influenced presumably by local somatic (epithelial?) tissue [91, 92].

Local influences are certainly operative in males. In the case of mouse testes lacking spermatogenesis because of a mutation in the *c-kit* gene or following treatment with busulfan (that denudes the testis of spermatogonia), infusions of spermatogonia acquired from neonates and from cryptorchid adult testes have similar chances of establishing themselves, but the immature pup testis is superior to the adult testis as a site for colonization and repopulation [93].

In adult male mammals, a one-way spermatogenic wave begins with the terminal DNA synthesis of GSCs [94] and ends with the completion of meiosis and differentiation of spermatids into spermatozoa. The spermatogenic waves of rat testis are set off simultaneously at as many as fourteen sites dispersed along the length of seminiferous tubules. In human beings, multiple onsets are not set off simultaneously and the “wave” is, therefore, not synchronized.

In “mammalian testes, various hypotheses have been proposed to describe the exact identity of GSCs and their pattern of division, most of which point to a subset of spermatogonia (Type A or a subtype of Type A) as GSCs ... Despite this, it has not been unequivocally shown whether GSCs in the mammalian testis divide asymmetrically” [95]. More advanced “spermatogonia” should probably be reclassified as TACs in the process of differentiating into spermatids and spermatozoa [96].

In addition to active spermatogonia, dormant or dark spermatogonia ( $A_d$  aka  $A_0$ ) would seem to play the role of reserve stem cells. They are beyond the reach of normal spermatogenesis but may become active in the wake of trauma and repopulate the seminiferous tubule with active spermatogonia [97].

#### **Vascular tissue (Blood and lymphatic tissue): Clonal hierarchies**

Vascular tissue, with cells suspended in plasma and lymph, occurs abundantly in loose CT as well as

in endothelial-lined vessels. Like CT cells, vascular tissue cells are immersed in extracellular material, but, unlike CT cells, vascular extracellular material is not typically or entirely of the cells own making.

Vascular cells include dividing cells resembling ASCs, RCs, and TACs. Although initially thought to originate in separate spleen and lymphocyte cell lines, one multipotent “stem cell” known as the hematopoietic stem cell (HSC) is now widely assumed to be the universal blood/lymphocyte precursor [98]. Over the years, notions of hematopoiesis have ranged from clones with a hierarchy of restricted “lineage potentials ... [to] a random process governed only by distributional parameters” [99].

Some clones exhibit multipotency. Amazingly, repeated plating of some cells reveals a hierarchy among the cell types differentiating, with primitive hematopoietic progenitors leading to spleen colony-forming units and granulocyte-erythrocyte-megakaryocyte-macrophage colony-forming units (CFU-GEMM). Granulocytes break out in three steps. Eosinophils and basophils arise from granulocyte precursors, while a neutrophil-monocyte line generates neutrophil and monocyte (macrophage) clones. Further down the line, bipotent megakaryocyte-erythrocyte progenitors gives rise to monopotent proerythroblasts that differentiate into erythroblasts and thence erythrocytes, and megakaryocyte-committed progenitors (MKP) that differentiate as megakaryocytes [100].

The unraveling of hierarchial clones stemming from HSCs began when Macfarlane Burnet borrowed the “clonal selection theory” from microbiology and suggested that colony-forming units (cells produced from a single progenitor) were the sources of specificity in the immune response [101]. Today, in addition to the inchoate sources of “effector cells,” long-lived memory cells are thought to persist and provide an enhanced response “after secondary antigenic stimulation” [102].

Burnet’s idea was quickly extended from lymphopoiesis to hematopoiesis generally, and Mako Ogawa and colleagues used a soft tissue culture medium (containing methylcellulose

conditioned by human lymphoblasts) to raise clones of suspended cells (from adult bone marrow, spleen, umbilical cord and elsewhere) [103, 104]. Serial plating of cells from these clones revealed that colony-forming units (dividing cells) were rare (e.g., one to two per  $10^6$  primary culture bone marrow cell) but could divide endlessly. These cells were vulnerable, however, to directed differentiation. Donald Metcalf discovered that interleukin 5 and stem cell factor (SCF, aka *c-kit* ligand or steel factor) increased the frequency of eosinophil-committed progenitor cells in multicentric colonies, and thrombopoietin with SCF increased the frequency of megakaryocyte-committed progenitor cells [105].

A consensus has yet to form around a nomenclature of blood cell and immunocyte dynamics. Such a nomenclature should insert a degree of rationality into the present hodgepodge of hemato/lymphopoietic terms. Lines should connect the dots of cell production, and cells at comparable levels of proliferation should be given comparable names. Some of today's terms should be retained: "common lymphoid progenitors" (giving rise to Pro-B, Pro-T, and Pro-NK [natural killer] lymphocytes) and "myeloid progenitors" (giving rise to macrophages, granulocytes, and dendritic cells) are useful terms [106]. But other terms should be dropped: "blast forming units" should be abandoned along with some of the "conveniences" frequently used to shorten expression such as "long term" and "short term stem cells". Histologists, hematologists, and immunologists will have a far better idea of what they're talking about when these changes are incorporated into the canon.

### Connective tissue (CT)

The chief cells of CT are fibroblasts (aka fibrocytes). Fixed fibroblasts are virtually dormant. They may, however, be mobilized as CC-like mesenchymal cells and be recruited through circulation to foreign sites where they may come to dominate connective tissue dynamics.

### Fixed CT

The most conspicuous feature of fixed CT is dormancy *in situ*. Retrospective birth dating

demonstrates that turnover in the connective tissue of the jejunum is extremely sluggish, taking fifteen to sixteen years [64]. Dormancy is lifted, however, under a variety of conditions indicating that constraints on cell division in fixed CT are imposed extrinsically and not through any intrinsic failure of fibroblasts. For example, the endometrial stroma of ovariectomized mice undergoes massive cell division when exposed to exogenous estrogen.

Dividing stromal cells are cache-like and should not be confused with ASCs. Thus, when endometrial stroma cells are labeled postnatally and prepubertally with the DNA analogue bromodeoxyuridine (BrdU), the number of label-bearing CT cells declines during the chase period and, indeed, only occasional "stromal LRCs were present after a 12 week chase. ... [Furthermore,] BrdU LRCs do not coexpress with Sca-1, a known stem cell marker, ... [and] stromal LRCs [a]re CD45- ... not CD45+ leukocytes of hematopoietic origin recruited from circulation [107]".

Dormancy is also lifted when fibroblasts are explanted to tissue culture. After a brief lag, the fibroblasts divide rapidly. Indeed, not only do they divide *in vitro*, but an underlying "feeder" layer of irradiated, non-multiplying fibroblasts is commonly employed to "condition" tissue culture media thereby aiding establishment and upkeep of more fragile cells, for example, epithelial and cancer cells [108].

Division of explanted fibroblasts in tissue culture has attracted a great deal of attention especially because of one peculiarity: it stops, on average, at a fixed number of divisions. The cells then enter a period of mitotic quiescence [109] that may last months but is eventually followed by death. Although many tissue culturists knew that fresh (aka primary) cultures of fibroblasts ultimately died, their failure to achieve cellular immortality was commonly attributed to culture conditions and not to any inherent property of cells. The phenomenon of cell mortality was only recognized after Leonard Hayflick showed that mitotic quiescence followed a predictable number of divisions conventionally known as the Hayflick limit. Indeed, cells exceeding their Hayflick limit "have cancer cell properties including higher rates of telomerase activity compared to normal cells" [110].

Of course, the possibility that organisms age and die when their fibroblasts reach their Hayflick limit is an alluring hypothesis [111] if a contentious one [112], but even fibroblasts from the elderly seem to have some unspent mitotic activity left when explanted to tissue culture. The mortality of organisms, therefore, would not seem to be a simple function of the mortality of fibroblasts.

### Mobile CT

Ironically, bone, the very symbol of stability, is remarkably dynamic and also the source of the astonishingly mobile medullary mesenchyme. Indeed, circulating 1,25-dihydroxyvitamin D<sub>3</sub> augments proliferation of bone-marrow stromal cells *in vitro* and increases the percentage of osteoblast-forming colonies (known as colony forming units or CFUs) formed by bone marrow cells *in vitro* [113].

Those suffering from osteoporosis, periodontal disease, arthritis, and osteolysis induced by tumors have firsthand knowledge of bone's dysfunctional dynamics, while those with healed fractures of bone are grateful for bone's functional dynamics. What is rarely appreciated is the massive amount of remodeling normally going on unbidden in bone. Normal bone "remodeling occurs in small packets of cells called basic multicellular units (BMUs), which turn bone over in multiple bone surfaces ... [A]t any one time, ~20% of the cancellous bone surface is undergoing remodeling" [114]. Beginning when *myf-5* and *myoD* genes are inactivated in osteocytes, bone remodeling is coordinated through intensely intimate interacting subroutines and climaxes when osteocytes become embedded in new matrix and lacunae are filled with new bone via osteoblast activity [115].

But the mobilization of bone elements reaches beyond bone remodeling and local dynamics. Like amoebas of the cellular slime mold, *Dictyostelium*, scrambling in concentric waves toward the source of cyclic-AMP [116], mobile CT, or mesenchymal cells move to areas recovering from injury and healing, and like *Dictyostelium* cells becoming fixed in a slug-like aggregate, mesenchyme becomes fixed in reforming CT.

Inevitably, the same factors implicated in normal local and systemic outreach have their pathogenic side. Unfortunately, osteoblastic recruitment is strongly stimulated in early human multiple myeloma. Osteoblasts produce large amounts of interleukin 6 (IL-6), the same potent myeloma cell growth factor that promotes bone-resorption [117]. Thus, a cytokine critical for the recruitment of osteoblasts and osteoclasts during bone remodeling is also "a potent myeloma cell growth factor" [118], and people with multiple myeloma suffer from painful bone resorption (osteolysis) resulting from high titers of IL-6.

### Adipose tissue

Finally, adipose tissue's place in CT is precarious. Adipocytes exhibit a range of mesenchym-like potentiality, and like fibroblasts *in vivo*, adipocytes resemble CCs by way of mitotic activity. But adipocytes' comparatively high metabolic activity suggests that they may not be CT cells in the first place. Adipose/smooth muscle metaplasia in uterine fatty tumors (UFT: lipomas and mixed lipoma/leiomyoma = lipoleiomyoma), suggests that adipocytes are more closely related to active smooth muscle cells than to lethargic fibroblasts [119]. The intimate association of adipocytes to capillaries likewise suggests a relationship to cellular (smooth) muscle rather than CT.

### Speculation on the evolution of tissues

Traditionally, animals are thought to have evolved either from aggregated amoeba-like cells that formed solid balls that differentiated internally or from hollow multicellular shells that folded in on themselves. Alternatively, Donald Williamson has proposed a different, non-linear, non-recapitulative theory of how animals evolved. According to Williamson, serial chimeras produced by multiple hybridizations stabilized through horizontal gene transfer gave rise to primordial animals [120]. These original types would then have evolved into phanerozoic animals through the filter of competition and natural selection.

A Williamson-like scenario assumes that isogametes of amoeba-like (amoeboid) and epithelial-like organisms conjugated during an age

before obstacles to hybridization were thrown up or thrown up as high as they are today. Inevitably, hybrids of ameba-like and epithelial-like organisms would have included organisms with different “ur-tissues.” These hybrids would then have been fair game for additional evolution.

Emergent chimeras would have had “ur-epithelia” (possibly epithelial-like pseudo-plasmodial aggregates of cells or a syncytial-like plasmodium) and “ur-connective tissues” of ameba-like cells (although pseudoplasmodia or a true plasmodia are equally conceivable). Subsequently, horizontal gene transfer would have brought genes together in new combinations allowing new interactions, and consequently the evolution of new tissues: “ur-muscle,” “ur-nerve,” “ur-germ” and “ur-hematopoietic” and “ur-connective” tissues. A paleoproterozoic biota might then have emerged of chimeric epithelial/cellular, epithelial/plasmodial, and/or epithelial/pseudoplasmodial organisms with circulating cells as well as specialized reproductive cells, and through mixing and merging genes and intra-organism competition, “ur-” tissue would have evolved into specialized and differentiated epithelia, muscle, nerve, and germ, hematopoietic and connective tissue.

One would expect that some ancient characteristics of the original organisms would be preserved in contemporary animals [121]. In sponges, an ur-ameboid tissue may be represented today by archeocytes (amebocytes); an ur-epithelium may be represented by collar cells (choanocytes) and pinacocytes; an ur-plasmodium may be represented by the syncytial exopinacoderm. But collar cells and pinacocytes do not quite qualify as epithelial, since they lack intercellular junctions (although septate junctions appear among sclerocytes) [122]. Division in archeocytes and collar cells may be cache-like, since mitosis is widespread and abundant when food is plentiful and ceases when food is sparse [123]. Similarly, the legendary ability of interstitial or ameba cells of some cnidarians and the neoblasts of planarians to mobilize and regenerate whole animals places these cells in the ranks of a pluripotential ur-ameboid tissue [124].

On the other hand, the disk-like placozoan, *Trichoplax*, hardly seems to consist of anything more than an ur-epithelium and possibly some muscle and nerve elements derived from it.

*Trichoplax* has a continuous epithelium differentiated into dorsal and ventral surfaces separated by an interspace containing a syncytium of mesenchymal “fiber cells” delaminated from the epithelium. “Since the fiber cells are contractile and may also be involved in the coordination of movement, they seem to combine the functions of muscle and neurons on a primitive level” [125]. All the epithelial cells are flagellated and joined apically by belt desmosomes while non-flagellated gland cells are interspersed among ventral epithelial cells. Division may be cache-like.

Were animals to have evolved from the result of unions between ameba-like and epithelial-like organisms, competition among “ur-tissue” within the organism may have provided the condition for the evolution of cellular dynamics in contemporary tissues. In effect, four of the tissues (epithelia, muscle, nerve, and germ) would have evolved chiefly from “ur-epithelium,” while two tissues (vascular and CT) would have evolved chiefly from “ur-connective tissue.” Some of their dynamic qualities would seem widespread, such as mitosis in CCs and CC-like cellular muscle, fibroblasts, and adipose tissue. Other qualities would seem to have evolved through convergent evolution such as the linearity of ASC, TACs, and TDCs and the hierarchy of HSCs and their progeny. Still other dynamic qualities may have evolved in parallel, for example, the CC qualities of proto-epithelia and fibroblasts and the RC qualities of skeletal muscle, germ, and memory cells in vascular tissue. Indeed, today’s tissue dynamics do not lose their grandeur were they to have evolved through competition and tradeoffs between the collective properties of epithelial-derived cells and the individual/social qualities of ameba-derived cells.

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