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Control of Cell Proliferation: Is the Default State of Cells Quiescence or Proliferation?

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Abstract

The control of cell proliferation in multicellular organisms remains a perennially controversial subject in experimental biology. In this essay, we examine the historical background and the rationale adopted by diverse theoretical and experimental research programs aimed at explaining *how* and *why* cells proliferate. We examine the premises that favor the notion that cells in multicellular organisms require direct stimulation from the outside (a task attributed to alleged growth factors) or from the inside (through the elusive action of oncogenes). Our analysis suggests that neither growth factors nor oncogenes directly stimulate the proliferation of cells. Based on evolutionary precedents, theoretical considerations and empirical data we posit instead that *proliferation* is the default state of all cells; thus, a search for extra- and intra-cellular inhibitory constraints promises to be productive when explaining this basic property of cells within the context of normal and abnormal developmental biology.

Keywords: proliferation, multicellular organisms, default state of cells, developmental biology

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Defining the Problem

From a historical and epistemological context, the biological sciences have evolved through two main basic theoretical foundations, namely, the cell theory and the theory of evolution. The cell theory posits that all organisms, be they unicellular or multicellular, are made up of cells and that multicellular organisms are generated from a single cell (Canguilhem 2008, Reynolds 2018). After overcoming criticisms regarding the place of syncytia and of individuality in the early 20th century, the cell theory remains unchallenged within the realm of biology at large (Harris 1999, Soto, Longo *et al.* 2016). Separately, Darwin's theory of evolution provided a coherent interpretation of how the many forms of life evolved (phylogenesis); it argues for common descent with modification and natural selection. Despite some course corrections to which Darwin's views have been subjected after the publication of the Origin of Species in 1859, such as the Modern and the Extended Evolutionary Syntheses, Darwin's contributions still remain as solid milestones in the history of evolutionary biology (Mayr 1982, Laland, Uller *et al.* 2014, Laland, Uller *et al.* 2015).

Notwithstanding these and other theoretical and empirical advances accomplished during the last century and a half, explanations regarding the control of cell proliferation in multicellular organisms remain controversial (Elsasser 1987, Noble 2012, Sánchez Alvarado and Yamanaka 2014, Longo, Montévil *et al.* 2015, Soto, Longo *et al.* 2016). For instance, a comprehensive explanation of how cell proliferation

is regulated in multicellular organisms and becomes integrated within the broader fields of cell, tissue and organ growth in size and shape is still lacking. In addition, epistemological and theoretical work aimed to resolve whether cell proliferation and motility are inducible or constitutive cell functions is still lacking as well. This essay will be dedicated to addressing these fundamental issues.

1. A Brief Historical Background

Toward the end of the second half of the 19th century, theoretical and empirical contributions by German pathologists solidified the role of cells in affecting healthy and diseased multicellular organisms while recognizing the interdependence of cells and the organisms to which they belong (Virchow 1960, Mayr 1982, Harris 1999, Sonnenschein & Soto 1999). This view was challenged at the beginning of the 20th century by three reductionist research currents. The first was the advent of genetics, which focused on the roles of genes in the phenotypes of organisms (Morgan 1910). The second was the introduction of cell/tissue culture into experimental biology as an important tool to study cell-based events (Willmer 1966, Sonnenschein & Soto 1999, Landecker 2007). Finally, the third current was the publication in 1914 of Theodor Boveri's book on carcinogenesis in which he posited that tumors were due to alterations in the structure of chromatin (considered by then to carry the genetic material) in a normal cell that would eventually become a cancer cell from which a tumor will grow in size and complexity by accruing mutated cells (Boveri 1914). Altogether, these three overlapping cell-based, bottom-up approaches (i.e., genetic determinism, cell culture and the somatic mutation theory of carcinogenesis) lead experimental biologists to adopt a cell-centered interpretative perspective of the living at large that became strengthened and hegemonic during the second half of the 20th century and which remains so to this day.

2. Is the Cellular Level of Biological Organization Alone Sufficient to Explain Morphogenesis?

From a single cell (the ovum), an adult multicellular organism evolves through a complex process. From early development to senescence, the process of organogenesis and its maintenance involves the interaction of different cell types within the many morphogenetic fields present in multicellular organisms. In most organs, those cell types are present in two distinct tissue types, i.e., the mesenchyme (which develops into adult connective tissue, a main component of the stroma, classically considered the support tissue of organs) and the parenchyma (classically considered as the functional, specialized part of organs). It is through those interactions that the shape and size of tissues, organs and systems are remodeled, repaired and regulated (Grobstein 1953, Howlett & Bissell 1993, Gilbert & Epel 2015, Cunha & Baskin 2016).

The reductionist turn alluded to above promoted the viewpoint that rigorous explanations of patterns of behavior happening at the tissue and/or organ levels of biological organization, such as proliferation, motility, and "differentiation", required a "mechanistic", bottom up, molecular description of processes happening within cells. In order to help in identifying the participants and their interactions during the processes of development, cell culture approaches appealed to researchers because they significantly reduce the number of variables present in animal-based experimentation. In the field of control of cell proliferation, cell culture offered the possibility of studying the cell cycle protagonists, their interactions and their dynamic properties while using hoped-for homogenous cell populations growing in glass or plastic culture dishes (Landecker 2007, Sánchez Alvarado & Yamanaka 2014, Pu, Han *et al.* 2020). Notwithstanding these intense efforts, an understanding of how cells control their reproduction remains undefined.

3. What do Cells do when Unconstrained?

Following the *Zeitgeist* established in textbooks and research publications on the subject, at the outset of our research program, ca. 1970, the consensus among researchers was that proliferative *quiescence* was the default state of metazoan cells (Bradshaw & Prentis 1987, Alberts, Bray *et al.* 1994). Consistent with this premise, in order to enter the cycle, cells would have required direct "stimulation" by either external (hormones and/or "growth factors") or internal factors

(oncogenes). Thus, despite accepting at the onset of our research program that *quiescence* was the default state of cells in multicellular organisms, empirical evidence we collected consistently contradicted it (Sonnenschein & Soto 1980). Specifically, the estrogen target cell lines we adopted as an experimental model proliferated in host animals only in the presence of estrogens, while in culture conditions they proliferated equally well regardless of the presence of ovarian hormones. After ruling out experimental errors, we still could not reconcile this paradox. To start with, we first wondered why biologists adopted proliferative *quiescence* as the default state for cells in multicellular organisms given that, in contraposition, microbiologists considered it axiomatic that the constitutive state of unicellular organisms was *proliferation* (see below). Altogether, after much empirical work, we concluded that *proliferation and motility* is the default state of all cells (Sonnenschein & Soto 1999, Soto, Longo *et al.* 2016, Sonnenschein & Soto 2020).

4. Searching for an Integrated Biological Context. A Theory of Organisms

Over the last decades, theoretical biologists expressed a need to complement Darwin's theory of evolution that addressed phylogeny with a theory that would explain ontogenesis (Polanyi 1968, Elsasser 1987, Woese 2004). This suggestion has received scant attention among biologists and thus, remained unfulfilled. Notwithstanding, theoretical foundations on the life cycle of organisms expanded and additional evidence accumulated in the field of control of cell proliferation. In collaboration with a group of colleagues in Paris, France, we identified three basic biological principles for a Theory of Organisms (Soto, Longo *et al.* 2016). Briefly, those principles are 1) the default state of proliferation with variation and motility (Soto, Longo *et al.* 2016), 2) the principle of variation, as the source of biological novelty and plasticity (Montévil, Mossio *et al.* 2016) and 3) the principle of organization, the source of robustness and stability (Mossio, Montévil *et al.* 2016, Montévil 2020).

In the current essay we are mostly focusing on the first of those principles, namely, the rationale behind our claim that the default state of *all* cells is *proliferation with variation and motility* (Soto, Longo *et al.* 2016). By virtue of being part of an interdependent system, during their lifetime, each cell in a multicellular organism is subject to a variety of exquisitely regulated controls that could either facilitate or prevent its proliferation. For instance, close structural contacts (among abutting cells) or interactions (through biochemical and/or biomechanical and bioelectrical forces) affect their proliferation and motility, as well as their metabolism, secretion and their overall phenotype (Sonnenschein & Soto 1999, Whited & Levin 2019).

5. The Control of the Proliferation of Individual Cells in Unicellular and Multicellular Organisms

Microbiologists who grew prokaryotic cells in a laboratory setting observed that in the presence of an adequate supply of nutrients, bacteria (prokaryotes) placed within permissive ranges of temperature, atmospheric pressure and pH, proliferated constitutively and exponentially (Luria 1975, Sonnenschein & Soto 1999). Later on, comparable patterns of *proliferation* were found when studying unicellular eukaryotes. Hence, among microbiologists, it became axiomatic that proliferation is a constitutive property of unicellular organisms; this constitutes their default state. This property not only applied to the microorganisms propagated in laboratories but, by extension, it also applied to the hypothetical first common ancestor of all living organisms, as well as all of its descendants. Arguments consistent with such views were already made by Malthus by the end of the 18th century (Malthus 1798), and later by Charles Darwin who, influenced by Malthus' views, inferentially strengthened the notion that *proliferation* was the default state of cells as documented by a passage in "The Origin of Species", namely "There is no exception to the rule that every organic being naturally increases at so high a rate, that, if not destroyed, the earth would soon be covered by the progeny of a single pair" (Darwin 1864). For the purpose of the current analysis, it then becomes relevant to ask... *Has the axiomatic default state of unicellular organisms remained unaltered through the advent of multicellularity to the present day?* So far, we have found neither theoretical nor empirical evidence that would challenge this axiom originally adopted by microbiologists (Sonnenschein & Soto 1999).

5.1. The Literal Adoption of Operational Terms: the Reification of Growth Factors

The success of microbiologists in culturing bacteria in a laboratory setting motivated other biologists to address comparable basic questions while using, instead, more complex, multicellular organisms. They found, unlike bacteria, that cells from metazoa required a more complex propagation medium containing macromolecules, like those present in serum, embryo extracts, etc. Even today, after considerable investments in designing so-called chemically-defined media, only a few cell lines can be routinely propagated in them. Components of those supplements were considered stimulators of cell proliferation, that is, the equivalents of "growth factors". Later, operationally defined "growth factors" were inferentially assumed to be real entities that indeed induced cell proliferation. Under this scenario, it was implicitly assumed that the default state of cells in metazoa was *quiescence* and that serum contained specific molecules (stimulatory signals) that stimulated (induced) cell proliferation. The term "growth factors" then acquired a narrow, regulatory meaning.

Starting in the 1950s, experimentalists began searching in earnest for stimulators of cell proliferation. Rita Levi-Montalcini, a biologist, and Stanley Cohen, a biochemist, were the first who characterized what eventually became known as a nerve growth factor (NGF) and an alleged epithelial growth factor (EGF), respectively. Levi-Montalcini, for her part, signaled all along that NGF did not stimulate the proliferation of nerve cells, but affected, instead, the number of neuron dendrites (Montalcini 1986). In contrast, Cohen and his followers insisted on claiming that EGF indeed stimulated the proliferation of cells (curiously, EGF mostly affected fibroblasts). Pragmatically, however, Cohen and his followers reached this conclusion when interpreting data showing an increased tritiated thymidine incorporation by cells in culture conditions, a method that falls short of actually measuring an increase in cell numbers (Carpenter & Cohen 1976, Cohen 1986, Sonnenschein & Soto 1999).

The rationale for claiming that "growth factors" could stimulate cell proliferation was curious. In a strategic reversal, the alleged physiological roles of "growth factors" in the whole intact animal were investigated after they were first purified. That is, instead of being discovered in the process of explaining a physiological function, like what happened with the discovery of insulin or estrogens, the strategy to discover "growth factors" consisted first in purifying a polypeptide from either serum, organ extracts or other complex natural sources and subsequently asking whether the suspected growth factor had indeed a physiological proliferative role when tested in culture conditions or administered to animals. For example, EGF was found, serendipitously according to both Cohen (Cohen 2008) and Gospodarowicz and Moran, in extracts of salivary glands of male mice during the purification of NGF (Gospodarowicz & Moran 1976). When these preparations were injected into newborn mice, they accelerated eye opening and tooth eruption. Intriguingly, both phenomena are related to epithelial cell death, rather than cell proliferation. Paradoxically, the cell line A-431, which was used to characterize EGF receptors, responds to EGF exposure by inhibiting cell proliferation (Barnes 1982).

Relevant references have shed both light and confusion on the subject. In the late 1970s, as an increasing number of novel alleged growth factors began to be described, Gospodarowicz and Moran listed a number of basic requirements that would have validated their presence (Gospodarowicz & Moran 1976). The requirements to qualify for becoming legitimate growth factors were 1) to initiate DNA synthesis; 2) to initiate one cycle of division in confluent cultures; 3) to trigger several cycles of division in sparse as well as confluent cultures; and 4) to generate clonal growth (starting from a single cell to a monolayer). Crucially, the specific evidence collected in culture conditions should have been matched by a comparable physiological proliferative role in animals. Other than the first of those requirements, i.e., to initiate DNA synthesis, the others remained unfulfilled. When one tests the function of a polypeptide, the control should not be the solvent, but instead should be a scrambled polypeptide containing the same amino acids with a random sequence. Additional objections could be raised. For instance, within a homeostatic context, nutrient starvation is not a valid alternative to evaluate the control of cell proliferation in a live animal. For instance, starved cells could have been taking up the polypeptides (EGF and others) added to the basic nutritive medium as welcomed supplemental nutrients needed to synthesize some DNA, but not enough to complete the final cell cycle

steps that Gospodarowicz and Moran alluded to as being required to fulfill their original growth factor definition. Also, proliferation rates in culture conditions in which those alleged growth factors were tested were either not exponential or showed no significant differences in cell proliferation rates (Carpenter & Cohen 1976, 1987). These inconsistencies between data on cells in culture conditions and physiological roles of alleged growth factors were noticed at the time by Renato Baserga, an experienced cell biologist, who nodded cautiously, "... this is not to say that reproduction *in vivo* is regulated by the same factors, but cell cultures are where we must start" (Baserga 1985).

Additional objections to the notion that the alleged growth factors directly stimulated cell proliferation were raised by others. For instance, EGF and TGF-alpha primarily stimulated cell spreading which, in turn, may have indirectly affected cell proliferation (Barrandon & Green 1987). Finally, toward the last decades of the 20th century, the advent of powerful recombinant DNA technology allowed for the use of species-specific recombinant polypeptides, and the generation of mice carrying null mutations (knockouts) of putative growth factors and their specific receptors. In the words of Durum and Muegge, the introduction of this technology provided the desired "acid test for the function of a gene" and consequently, claims emanating from data gathered in culture could be reliably tested (Durum & Muegge 1998). The data collected, however, failed to show that those alleged growth factors singly or in combination had a direct role in the control of cell proliferation (Miettinen, Berger *et al.* 1995, Sibilia & Wagner 1995, Threadgill, Dlugosz *et al.* 1995, Guo, Degenstein *et al.* 1996). Reports concluded, instead, that these alleged growth factors were either i) "survival factors", or cell death inhibitors (Koury & Bondurant 1988, Williams, Smith *et al.* 1990), ii) made cells spread (Barrandon and Green 1987), or iii) affected cell differentiation that was unrelated to the control of cell proliferation. These alternative conclusions to those reached by Stanley Cohen and his followers fit well within views that once cells are placed in an environment where nutrients are in adequate supply, in the absence of *bona fide* inhibitors, they exercise their constitutive ability to proliferate making stimulation moot (Sonnenschein & Soto 1999).

A clarification is in order: the data stemming from work in developmental biology suggest that these polypeptide alleged growth factors may indeed play roles as morphogens (Gilbert 2013). In this essay dedicated to defining *the how* and *the why* in the control of cell proliferation, however, we are merely challenging the notion that these polypeptides have instructive properties for cells to enter the cell cycle in living organisms. The answer is that they do not (Cohen 1965, pp. 251-272, Gospodarowicz & Moran 1976, Cohen 1986).

Equally baffling have been claims of endogenous stimulators of cell proliferation (oncogenes) by proponents of the somatic mutation theory of carcinogenesis (Huebner & Todaro 1969, Tabin, Bradley *et al.* 1982, Bishop 1991, Varmus & Weinberg 1992, Malumbres & Barbacid 2009). In fact, an extended volume reportedly aimed at reaching a consensus about the stimulatory role of growth factors and oncogenes on cell proliferation dealt, instead, with intracellular biochemical interactions triggered by so-called growth factors and oncogenes rather than with verifying the biological role (increased cell numbers) of those extra- and intracellular alleged stimulators of cell proliferation (Bradshaw & Prentis 1987). A comparable conflation between the notion of control of cell proliferation and activation of signal transduction is still observed in current publications (Lavoie, Gagnon *et al.* 2020). Meanwhile, contemporaneously published textbooks and research articles retain the notion that *quiescence* is the default state of cells in multicellular organisms and that growth factors and oncogenes directly stimulate the proliferation of cells (Alberts, Johnson *et al.* 2008, Weinberg 2014).

6. The Why and the How of Cell Proliferation in Multicellular Organisms

During the diverse stages of development, some cell types proliferate while others do not, regardless of their location in the organism and their differentiated function. Instead, when placed in culture conditions, explants originating in cell populations that are mostly dormant in animals proliferate robustly (for instance, fibroblasts) (Hayflick 1992). In the early 20th century, this cell behavior was interpreted as equivalent to having been "des-inhibited" from a proliferative inhibition exerted while inside multicellular organisms (Carrel 1912). By adopting the premise that, under

homeostatic conditions, proliferation and motility is the default state of all cells, it becomes implicit that a cell's metabolic or secretory activity and its phenotypic changes appear to be not relevant when answering the question, *why* do cells proliferate?

6.1. How do Cells Proliferate?

"Established" cell lines have been extensively used for the study of the phases of the cell cycle; they better tolerate nutrient starvation, metabolic poisoning, extreme environmental temperatures, or exposure to undue physical stress. Under these experimental conditions, cells in culture can be prevented from proceeding with the cycle stages. Meanwhile, as alluded to above, molecular interactions taking place along the cell cycle phases can be explored in order to answer the question, *how* do cells proliferate? In fact, other than those estrogen and androgen target cells that we worked with there is a severe paucity of "physiological" means of synchronizing cell populations growing in culture conditions. To remedy this shortcoming, experimentalists had adopted non-physiological methodologies (e.g., nutrient starvation, poisons, etc.) in order to synchronize cell populations. This has been the preferred strategy to define the successive steps and pathways that cells take in order to generate two daughter cells from the metaphoric mother one (Min, Rong *et al.* 2020). Indeed, by 1990, Paul Nurse, who used both unicellular eukaryotes (yeast) and cells from multicellular organisms already concluded that "...A case can now be made for the existence of a universal control mechanism common to all eukaryotic cells" (Nurse 1990). In this context, answers to the *how* question became linked to the role played during the cell cycle by enzymes, cyclins, transcription factors and other components present in and within the cell's plasma membrane.

Soon after Nurse made this generalization, this field of research exploded with descriptions of the myriads of biochemical interactions occurring during the phases of the cell cycle of all types of eukaryotic cells. Dozens of alleged oncogenes and proto-oncogenes like the transcription factor Myc and families of enzymes operating during the cell cycle, such as mTOR kinases and others have been shown to participate in these interactions (Bradshaw & Prentis 1987, Hunter 1998, Malumbres & Barbacid 2001, Gabay, Li *et al.* 2014, Sever & Brugge 2015, Lavoie, Gagnon *et al.* 2020, Liu & Sabatini 2020). Altogether, the answers to the *how* question have provided a rich catalogue of participants interacting during the diverse phases of the cell cycle, a biochemical catalogue that keeps expanding and will continue in the foreseeable future.

6.2. Why do Cells Proliferate?

Returning to the question related to the control of cell proliferation formulated along the lines of *"why* does a cell proliferate?" it is necessary to *a priori* adopt a premise that would address the issue of the default state of cells, that is, *what do cells do when unconstrained?* Evidently, when researchers adopt a given premise, it represents a major theoretical commitment because such a choice determines what is to be explained and thus it necessarily guides research in a particular direction. For example, if one were to adopt proliferative *quiescence* as a valid premise, what needs to be explained in this context is what makes cells not be quiescent, that is, what makes them proliferate. As mentioned above, when microbiologists axiomatically acknowledge that the default state of unicellular organisms is proliferation, they do not need to search for stimulators. Counterintuitively however, for over a century, experimentalists working with cells from multicellular organisms have adopted *quiescence* as the default state of those cells. Therefore, they focused on identifying and characterizing alleged stimulators of cell proliferation.

What has been the traditional narrative in textbooks and research articles in this field regarding the *how* and *why* questions? These two highly relevant discrete questions have been either ignored altogether or were amalgamated into a single one, namely, *how* does a cell proliferate? (Malumbres & Barbacid 2001, Alberts, Johnson *et al.* 2008, Cross, Buchler *et al.* 2011, Hunt, Nasmyth *et al.* 2011, Weinberg 2014, Sever & Brugge 2015, Novák, Heldt *et al.* 2018, Liu, Michowski *et al.* 2019, Liu & Sabatini 2020)

6.3. Does the Empirical Evidence Support the Principle that the Default State of All Cells is Proliferation?

Estradiol-17beta target cell lines have been a reliable experimental model for assessing our claim that proliferation is the default state of cells. In serumless

medium, estrogen-target cells proliferate exponentially in the absence of estrogens. Meanwhile, the addition of estrogen-less serum inhibits their proliferation in a serum concentration dependent fashion (Soto & Sonnenschein 1985); physiological concentrations of estrogens cancel this inhibition. Another relevant example of proliferative control is represented by the role of erythropoietin in the regulation of the number of erythrocytes in the bloodstream; here, erythropoietin acts by inhibiting cell death and thus allowing for the constitutive proliferation of erythroid precursors to be expressed (Koury & Bondurant 1988, Williams, Smith *et al.* 1990). Additional experimental examples buttressing *proliferation* as the default state are the inhibition of fibroblast proliferation by homologous serum (Sonnenschein & Soto 1981), the "ground-state" of embryonic stem cells (Ying, Wray *et al.* 2008), the active induction of proliferative *quiescence* in lymphocytes (Yusuf & Fruman 2003), and the constitutive proliferation of epithelial cells of Hydra during starvation (Bosch & David 1984).

An additional helpful hint to decide whether the default state is either *proliferation* or *quiescence* is provided by the adoption of an evolutionary perspective on the subject. For centuries, naturalists and biologists have widely recognized a common property of living objects that distinguishes them from the inert; this property was their ability to generate actions, exemplified by their ability to proliferate and move, and to create their own rules, particularly the aim of maintaining themselves alive. This property is called normative agency (Soto & Sonnenschein 2018). As mentioned above, regardless of how the first cell (or protocell) was generated, it stands to reason to assume that about 3.8 billion years ago, in the midst of a prebiotic soup, such a cell must have had the constitutive property to proliferate and move. From an evolutionary perspective, the generation of multicellular organisms from unicellular eukaryotes involved the conservation of previously existing levels of organization (Nurse 1990, Sonnenschein & Soto 1999). The constitutive capacity of cells to proliferate within a multicellular organism must have remained unaltered and hence, their default state conserved. As mentioned above, this idea is supported by the high homology between the cell cycle effectors of yeast and human cells (Nurse 1990, O'Farrell 2011).

Additional arguments buttress the need for an overdue reassessment of the default state of cells in multicellular organisms. For instance, in multiple species embryos develop outside of the parental organisms, demonstrating that exponential proliferation in early development may take place in sea water (urchins) in the absence of alleged growth factors (Nesbit, Fleming *et al.* 2019). Later during development, as different tissues are formed, proliferation is distinctively regulated suggesting that, with the emergence of multicellularity, inhibitory controls impose an induced quiescent state upon different cells in specific tissues. Once these cells become "freed" from organismal restraints, they manifest their default state by proliferating, as they do when explanted into routine culture conditions.

Conclusions

For over a century, research on cellular biology has been conducted under the premise that *quiescence* is the default state of cells in multicellular organisms (plants and animals). In contrast, microbiologists axiomatically acknowledge that the default state of unicellular organisms is *proliferation*. Moreover, no cogent argument has been offered so far that would justify a radical switch of the ancestral default state of cells with the advent of multicellularity. Notwithstanding these theoretical and empirical arguments, proliferative *quiescence* remains at the core of teaching at all levels of education and of research projects in developmental biology and as a basic premise of the currently hegemonic theory of carcinogenesis, i.e., the somatic mutation theory. Our analysis of this situation suggests that the adoption of this wrong premise might be responsible for the conceptual confusion in the fields of a) developmental biology, especially about how size and shape of tissues and organs are regulated and b) carcinogenesis. It follows that a radical theoretical change in biological thought is necessary regarding how the control of cell proliferation is regulated; this reassessment should contribute to resolving this crisis. As presented above, evolutionarily relevant alternatives are available and supported empirically. They rely on adopting *proliferation* as the default state of *all* cells.

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