The Evolution of Interdependent Cell Cycles During the Transition to Multicellularity

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Abstract

In the evolution of complex multicellular organisms, cells that were once autonomous became obligately dependent on one another for survival and reproduction. Despite its importance, the process by which autonomous cellular machinery was restructured into obligately interdependent networks is poorly understood. Addressing this gap requires a framework that clearly categorizes the different levels of interdependence and identifies how the ancient, conserved eukaryotic cell-cycle machinery of unicellular species was modified so that cell survival and reproduction became conditional on other cells. Here, we first examine the cellular autonomy in unicellular organisms and how cell fitness in these organisms can be influenced by interactions with other cells, revealing ancestral mechanisms that may have been co-opted during the transition to multicellularity. Second, we trace how an increasingly interdependent cell network emerged, from undifferentiated multicellular organisms in which all cells maintain their reproductive ability to organisms with reversible and irreversible germ-soma differentiation. Central to this transition is understanding how cell cycle machinery was modified to integrate extracellular signals and enable the inheritance of cellular states across the cell cycles. Finally, we propose a framework to structure comparative studies of how cellular autonomy was progressively restricted during the repeated, independent origins of obligate multicellularity.

Introduction

Cells in obligate multicellular organisms depend on each other for their survival and reproduction, unlike ancestral unicellular organisms, where cells can survive and reproduce on their own. Obligate multicellularity has evolved independently from unicellular ancestors numerous times over Earth's history (Grosberg & Strathmann, 2007; Lamża, 2023). The stepwise process by which cells became dependent on one another during the transition to obligate multicellularity remains a key unanswered question in evolutionary biology.

Dependency is defined as a one-way interaction when one cell depends on the other for its survival or reproduction. Interdependency, on the other hand, is the situation when both cells depend on each other for their survival or reproduction. Survival and reproduction are the processes that constitute the components of cellular fitness in which various levels of dependency can arise. An extreme case of interdependency is observed in many animals and plants, where cell survival depends on continuous signals from neighboring cells and the surrounding tissue (Alberts et al., 2002; Liu et al., 2017). When these signals are withdrawn, cells activate programmed cell death pathways, removing cells that are no longer needed or potentially harmful (Raff, 1992). This creates an obligate interdependency among cells: survival is restricted to those that are correctly positioned, appropriately connected, and functionally integrated within the organism. In this context, a cell's survival is conditional on its contribution to the multicellular system. Another example of dependency is in organisms with separation of soma (vegetative) and germ (reproductive) cells (Swartz & Wessel, 2015). In these organisms, germ cells rely on soma for their survival and somatic cells rely on germ cells to transmit their genes to the next generation. Obligate cellular interdependence for organismal reproduction is often associated with mechanisms that limit somatic cell proliferation and establish stable cellular identities, which reduces their capacity for regeneration (Libby & Ratcliff, 2014; Ruijtenberg & van den Heuvel, 2016).

These extreme cases of cell-cell interdependency which are often accompanied by cellular specialization require mechanisms that allow cells to maintain their identity by preserving cellular memory throughout an organism's life cycle (Ringrose & Paro, 2004; Stewart-Morgan et al., 2020). For example, faced with an environmental stimulus, differentiated cells in a multicellular organism need to mount a coordinated response (or no response) by sending, receiving, and processing specific signals to adjust each other's survival, growth and

division (van Oosten-Hawle & Morimoto, 2014). The molecular machinery that enables such intercellular communication and regulation in eukaryotes are ancient and conserved across both unicellular and multicellular organisms (Chantranupong et al., 2015; Morgan, 2007). However, during the transition to obligate multicellularity, mechanisms that preserve cellular state memory and communication must have been integrated with the ancestral system. A key question is how the autonomous unicellular machinery progressively became more reliant on cellular memory maintenance and cell-cell communication to produce highly complex and interdependent cellular networks in multicellular organisms.

In this paper, we outline the organizing principles with which to understand the progressive increases in cellular interdependency during the transition to multicellularity and propose a framework for studying its evolution. We first evaluate autonomy and dependency among cells in unicellular eukaryotes. Second, we examine cell-cell interdependency in multicellular organisms, including organisms with undifferentiated multicellularity, with reversible germ-soma differentiation and organisms containing a dedicated germ and irreversibly differentiated soma. Third, we explore key molecular mechanisms that are likely necessary for the evolution of interdependency, and their possible ways of integration with the conserved unicellular machinery. Finally, we outline how the concept of interdependency can be used to gain deeper insight into evolution of mechanisms required for obligate multicellularity.

Autonomy and dependency in survival, growth, and division in unicellular eukaryotes

A unicellular organism in isolation is expected to harbor all the mechanisms required to survive, grow and divide on its own and rely solely on the cell's internal state and external abiotic conditions to maximize its own fitness. However, unicellular organisms live in populations with other cells that they interact with. Here we explore whether a cell's fitness in unicellular organisms can depend on the presence of other cells in its environment. Variation in the behavior of unicellular organisms at different population densities offer clues into the earliest signals that may have contributed to the evolution of multicellularity.

The survival of unicellular organisms can depend on a minimum population density

In optimal environmental conditions, the survival of unicellular organisms is not expected to depend on a minimum population density. This seems to be the case for most unicellular species as they can be cultured as single cells if the medium is sufficiently nutrient-rich. However, if a unicell's fitness depends on a minimum density of conspecific cells (Ohkawa et al., 2020), this might hint at the existence of specific cell-cell interactions prior to the evolution of multicellularity and provide insights into the ancestral mechanisms for the evolution of cell interdependency in multicellular organisms.

Cases of interdependency in survival for unicellular organisms are extremely rare. An example of this comes from the cultures of the free-living ciliate, *Tetrahymena thermophila*, in which cells undergo a rapid death if the culture density goes below a minimum threshold because cell survival is regulated through the release of autocrine factors (Christensen et al., 2001). Autocrine factors are signals that a cell produces which affect the cell itself (Su et al., 2024). When cells are in liquid, this signal rapidly diffuses away from the cell and makes cell survival dependent on a minimum population density to maintain this autocrine signal at a high enough concentration (Cassidy-Hanley, 2012; Christensen et al., 2001). Autocrine signaling is also found in multicellular organisms, where it contributes to coordination between cells and can generate functional interdependence between cell types (Doğaner et al., 2016). Thus, autocrine signaling in unicellular organisms may be a precursor to cell-cell communication molecules adopted by multicellular organisms (Miller & Bassler, 2001). Studying autocrine signaling in unicellular organisms may provide important insights into the evolutionary origins of cellular interdependency (Christensen et al., 1998; Tong et al., 2017).

A more common form of density-dependent survival among unicellular organisms occurs under stress. Stress responses typically require metabolic investment that competes with growth and division, creating a trade-off between survival and reproduction (Nyström, 2004; Zakrzewska et al., 2011). For example, in *Chlamydomonas reinhardtii*, a green alga, reproduction occurs through multiple fission where 2^n (n typically between 2 to 5) cells are produced at every round of division and kept in a mother cell wall until the daughter cells develop flagella, digest the cell wall and disperse (Bišová & Zachleder, 2014). Under stress, daughter cells delay digesting the mother cell wall and stay together, forming a structure

called a palmelloid (De Carpentier et al., 2019; de Carpentier et al., 2022). Cells in these palmelloid structures appear to coordinate their release so that they all delay the formation of flagella and release of the cell wall digesting enzyme together. Investigating if there are mechanisms that allow coordination among cells in these small groups can be relevant to understanding the evolution of early stages of dependency on other cells to enhance survival.

Changes in unicellular behavior at high cellular density

Since cells in multicellular organisms live in close contact with other cells, evaluating the unicellular behavior under high cell density may provide clues to the evolution of interdependency in multicellular organisms. As cellular density increases, and the microbial population reaches a stationary growth phase, cell populations often show a change in behavior which can stem from two factors (De Virgilio, 2012). High cell density can cause a change in the behavior of unicellular organisms due to the accumulation of specific cell-cell communication molecules called quorum-sensing (Chen & Fink, 2006; Hornby et al., 2001), or can occur due to changes in the common environment, for example due to nutrient depletion and waste accumulation (Galdieri et al., 2010; Werner-Washburne et al., 1993).

Quorum sensing, a mechanism of cell-cell communication via secreted, hormone-like signaling molecules, enables cells to detect population density that can synchronize their behaviors. Though quorum sensing is most commonly associated with bacteria (Waters & Bassler, 2005), it has also been detected in numerous eukaryotic microbial species and directly demonstrated to induce switches in normal or filamentous growth in the budding yeast *Saccharomyces cerevisiae* and the commensal pathogen *Candida albicans* (Albuquerque et al., 2013, Chen & Fink, 2006; Hornby et al., 2001). Such density-dependent signaling mechanisms illustrate how individual cells can regulate their response to signals produced collectively by neighboring cells. These mechanisms provide insight into how the decisions of single cells can become dependent on local cell populations, a principle that underlies the regulation of proliferation, differentiation, and survival among interdependent cells in multicellular organisms.

The environmental consequences of high cellular density, although not due to cell-cell interactions, might provide the conditions for development of cell-cell dependencies. In contrast to spatially homogenous liquid environments, growth on solid media creates spatially

structured environment where neighboring cells are often related and in turn promote the evolution of cooperative behaviors (Nadell et al., 2010, 2016). One potential example is the induction of programmed cell death in cells with limited access to nutrients. If such cells die through necrosis, they may release harmful cellular contents that disrupt neighboring cells. In contrast, programmed cell death can limit this damage and may even release nutrients that benefit surrounding cells (Durand et al., 2011, 2014). In dense clonal populations, mechanisms that trigger controlled death of severely damaged cells could therefore benefit neighboring, likely related, cells. The importance of controlled cell death, apoptosis, is well-recognized for proper functioning of multicellular organisms (Huettenbrenner et al., 2003). Analogous cooperative behaviors in clonal cell clusters may represent early evolutionary steps towards cell-cell interdependence where the survival of individual cells becomes linked to the survival of the group.

Increasing interdependency in survival, growth, and division in the transition to multicellularity

The transient benefits of cell-cell interactions in unicellular populations—such as maintaining autocrine signals above a critical concentration—can become permanent when genetically identical daughter cells remain physically attached after division. Because clonal attachment creates a clear boundary between self (the group) and non-self (unrelated cells), it satisfies the conditions for the evolution of altruistic behaviours: costly actions that benefit group members are directed exclusively toward relatives who share the same genes (Hamilton, 1964 I & II). Cell-cell attachments that keep daughter cells together while excluding unrelated cells are therefore a critical step in the evolution of interdependent cell networks (Abedin & King, 2010; Fisher et al., 2013).

Stable cell-cell connections might provide the possibility for an early division of labor where cells can specialize in the production of different metabolic products, such as enzymes, which would enhance their access to a wider variety of nutrient resources (Ispolatov et al., 2012; Pfeiffer & Bonhoeffer, 2003). Cell division requires a complete remodeling of the cell's cytoskeleton, and if specialization in a certain task becomes incompatible with division, certain cells might become dedicated to survival and growth processes and others to reproduction, leading to division of germ and soma (Cooper & West, 2018; Koufopanou, 1994).

Germ cells are involved in reproduction, both asexual and sexual. In asexual reproduction, the germ cells can regrow the entire organism. In sexual reproduction, germ cells produce gametes through meiosis and the resulting cell from the fusion of gametes, the zygote, can reproduce the entire organism. Somatic cells' ability in reproduction is restricted to various degrees. Certain somatic cells cease division for the rest of the organism's life cycle and perform a specific task and are called terminally differentiated cells. Organisms with repair and regeneration throughout their life contain a second category of somatic cells called stem cells (Weissman, 2000). Stem cells continue division, but they can only produce a limited set of cell types and their division capacity is tightly regulated by extracellular signals so that they only reproduce when and where they are needed. Here, we explore multicellular organisms in the order that cells progressively lose their autonomy and become dependent on the cell network in their survival and reproduction.

Interdependency in undifferentiated multicellular organisms

Undifferentiated multicellular organisms are composed of cells with no apparent morphological specialization. These organisms might be the first step in stabilizing cell networks that can make the evolution of division of labor possible. In undifferentiated multicellular organisms all cells are thought to retain equivalent reproductive and metabolic capabilities (Grosberg & Strathmann, 2007). These are phenotypically closest to the transient, high-density groups formed by unicellular organisms under stress (Olson, 2013) with the key difference being that daughter cells stay together through cytoplasmic connections after division (Chaigne & Brunet, 2022).

These organisms are found mostly among the Volvocine green algae, such as *Tetrabaena socialis* and *Goinium pectorale* (Featherston et al., 2018; Hanschen et al., 2016). It is relatively easy to understand the formation of these multicellular groups through a set of changes in the ancestral unicellular division program (Kirk, 2005). Similar to *C. reinhardtii*, these organisms also undergo multiple fission, but each round of mitosis ends with incomplete cytokinesis. The incomplete cytokinesis creates an exclusive bond between daughter cells which is later replaced by an extracellular matrix (Arakaki et al., 2017). Whether cytoplasmic bridges act as only a mechanism that physically links daughter cells, or it also serves as a mechanism for transferring substances exclusively to the genetically

identical cell is unknown (Chaigne & Brunet, 2022). Cells also maintain a specific angle of division relative to one another which produces the specific colony architecture (Baena-López et al., 2005; Scheres & Benfey, 1999). Cells in these groups are coordinated in their function and invest in survival and reproduction in a temporal manner where all cells first perform a function, such as swimming, and then start growth and division (Kirk, 2003). The colony size is determined by mother cell's growth size, which is determined by the environmental condition and the maximum growth size possible (Craigie & Cavalier-smith, 1982; Jong et al., 2021).

These organisms face the same issue as unicellular organisms by having to choose between survival or reproductive functions temporally. The advantage offered to each cell by staying in a group could be due to an increase in size, better protection from environmental stress by the extracellular matrix or sharing resources through cytoplasmic bridges or in the extracellular matrix (Grosberg & Strathmann, 2007; Pfeiffer & Bonhoeffer, 2003).

Organisms with reversible differentiation

In undifferentiated multicellular organisms, all cells have full regenerative potential (totipotent). Organisms with reversible differentiation, by contrast, contain specialized somatic cell types, yet retain regenerative capacity through populations of stem cells that can reconstitute the entire organism. Most importantly, these organisms usually lack a permanently sequestered germline. As a result, the relationship between individual cells and their contributions to survival and reproduction is flexible and can be reconfigured (Extavour & Akam, 2003; Lanfear, 2018; Radzvilavicius et al., 2016). No single cell in these organisms can, without experimental manipulation, regenerate the whole organism. While certain cells possess regenerative capabilities, this depends on their integration within a network of interacting cells that provide structural, chemical, and positional information (Jones & Wagers, 2008; Scadden, 2014). From an evolutionary perspective, the cellular network itself may have preceded division of labor, enabling the emergence of graded regenerative capacity. It is therefore most productive to understand regeneration as an emergent property of the cellular network rather than as a property of isolated cells.

Organisms with reversible differentiation are found across the tree of life. In a mature vascular plant, leaf and root cells serve survival functions while floral organs are responsible

for reproduction, yet this division is not permanent. The shoot apical meristem can give rise to either leaves (survival) or flowers (reproduction), and in perennial plants some meristems remain vegetative while others undergo the floral transition, allowing the plant to regenerate its reproductive capacity season after season (Albani & Coupland, 2010). A comparable flexibility exists in the cnidarian, Hydra, whose body is maintained by three distinct stem cell populations: ectodermal epithelial, endodermal epithelial, and interstitial stem cells (Bosch, 2007). In a Hydra, epithelial cells are responsible for structure and contraction (survival) and interstitial stem cells can give rise to germ cells (reproduction). However, when Hydra is bisected, each fragment regenerates a complete organism, remarkably, even animals experimentally depleted of all interstitial cells regenerate and reproduce normally because epithelial cells themselves function as self-renewing stem cells (Chera et al., 2009; Marcum & Campbell, 1978). In both organisms, the boundary between somatic and reproductive contributions can be redrawn depending on the context. This plasticity illustrates a key property of organisms with reversible differentiation, that the survival-reproduction network can be rebuilt from a subset of cells, provided those cells retain access to the positional and signaling cues of the tissue environment.

Organisms with irreversible germ-soma differentiation

At the most exteme end of the interdependency spectrum, organisms with irreversible germ-soma differentiation, permanently partition reproductive capacity. The germline is typically sequestered early in development (Seydoux & Braun, 2006; Strome & Lehmann, 2007), and the distinction between germ and soma is actively maintained by regulatory programs where germ cells repress somatic differentiation, while somatic cells suppress reproductive gene expression (Cinalli et al., 2008; Seydoux & Braun, 2006). Although some somatic cells retain limited regenerative potential, for example, through tissue-specific stem cells, they cannot reconstitute the whole organism. In these organisms, the contribution of different cell types to survival and reproduction is fixed.

All somatic cells depend on germ cells for transmission to the next generation but the degree to which germ cells depend on somatic cells varies. In the volvocine green algae, *Volvox carteri*, the simplest organism with a complete germ-soma division of labor, somatic cells are specialized for flagellar motility, incapable of dividing, and programmed to senesce within days (Hallmann, 2011; Kirk, 2001). The germ cells (gonidia) are nonmotile and rely on

somatic cells both for locomotion, essential for phototaxis and the advective nutrient transport generated by flagellar beating, without which metabolite supply to the colony interior would be insufficient (Koufopanou, 1994; Solari et al., 2006). This mutual dependency is established during embryogenesis through asymmetric cell divisions and is maintained by the *regA* gene, which most likely represses chloroplast biogenesis in somatic cells, preventing them from growing and reproducing (Kirk, 1997).

In organisms with multiple somatic cell types, dependencies become more complex and hierarchical. Some somatic functions are indispensable for germ cell survival, for instance, circulatory and respiratory systems sustain the oxygen and nutrient supply on which all cells depend while others are dispensable or redundant. The existence of tissue-specific repair mechanisms through stem cell populations adds further layers of dependency among somatic cells themselves. Thus, germ-soma segregation in complex organisms creates a hierarchical survival dependency network in which some cell types are more critical than others for maintaining the conditions under which germ cells can survive and reproduce. Categorising and catelogging these dependencies will help illuminate how interdependencies evolved in complex multicellular organisms.

Evolution of mechanisms of interdependency

Many components that control eukaryotic cell survival and division, including protein regulators of cell cycle, such as cyclin-dependent kinases, cell cycle checkpoints and programmed cell death pathways, are conserved between unicellular and multicellular organisms (Cross et al., 2011; Harashima et al., 2013). This means that the molecular mechanisms required for establishing survival and reproductive dependency networks in the transition to multicellularity involved co-option and modification of pre-existing toolkits (King, 2004; Olson & Nedelcu, 2016; Love and Wagner 2022 Evolution).

The conserved eukaryotic cell cycle contains several decision points that become targets of evolutionary modifications during the transition to multicellularity. In unicellular organisms, entry into the cell cycle (G1) and commitment to DNA replication at the G1/S transition are governed primarily by nutrient availability and internal cues, such as cell size (Johnson & Skotheim, 2013). Growth control is mediated by deeply conserved nutrient-sensing pathways, particularly the Target of Rapamycin (TOR) pathway, which promote cell growth in response

to amino acids and energy across all eukaryotes (Ahmad et al., 2019). During the evolution of multicellularity, an additional layer of regulation was superimposed on this ancestral machinery by integrating extracellular signals from growth factors and neighboring cells, which often act by modulating nutrient uptake and TOR activity (González et al., 2020). As a result, progression through the cell cycle is no longer autonomous, but conditional on the state of the cell itself and the signals received from surrounding cells.

A central component in G1/S transition is the Retinoblastoma (RB) pathway whose function might have been subjected to rewiring during the evolution of multicellularity. In unicellular organisms such as *C. reinhardtii*, the RB homolog functions primarily as a size checkpoint that links cell size to division number during multiple fission (Goodenough et al., 2007). In multicellular organisms, the same pathway has become a point of integration for extracellular signals, as well as a cell's internal conditions by controlling access to DNA replication, ensuring that cells only divide when they are needed by the entire organism. Strikingly, co-option of the RB pathway appears to have been formative in the early steps of the evolution of multicellularity in volvocine green algae (Hanschen et al., 2016). The RB pathway thus illustrates how a cell-autonomous pre-existing cell size sensor can be re-purposed to enforce socially regulated division in multicellular organisms.

A critical feature defining cellular networks with division of labor is for cells to not only obtain new roles but to maintain them throughout the organism's life cycle. This stability in cell identity depends on the ability of cells to maintain a form of memory of their epigenetic state across time and cell divisions. In unicellular organisms, gene expression states are often flexible and can change quickly in response to the environment. However, mother cells can pass on a transcriptional memory to the daughter cells, enabling them to restart growth immediately (Xue & Acar, 2018). In multicellular organisms, cells must maintain consistent identities even as conditions change. This is achieved mainly by reinforcing gene expression patterns through positive feedback loops (Xiong & Ferrell, 2003) or chromatin modifications (Ringrose & Paro, 2004). Epigenetic mechanisms, such as chemical modifications to DNA and chromatin, help stabilize these patterns and allow them to be inherited during cell division. Finally, signals from neighboring cells also contribute by continuously reinforcing appropriate states within cells. These changes can transform an autonomous regulatory system into components of an interdependent network where cellular behavior is controlled at the level of the organism rather than individual cells.

A framework for studying the evolution of cellular interdependency in the transition to multicellularity

This paper set out to address two linked questions: first, how did cells that once survived and reproduced autonomously become obligately dependent on one another; and second, how was the ancestral cell-cycle machinery progressively rewired, transforming independently operating cells into coordinated, interdependent networks? The perspective presented here provides a unifying way to compare diverse multicellular systems without assuming a single evolutionary trajectory. Rather than categorizing multicellular organisms into broad groups such as simple and complex, this framework encourages analysis of which aspects of autonomy are restricted, by what mechanisms and with what temporal and spatial stability. A set of guiding question can help with structuring studies on this topic (Table 1).

A mixture of evolutionary and cell molecular biology techniques need to be used to get a comprehensive view of the evolution of interdependent cell cycles. Among these are comparative genomics approaches in which a genome of a single-celled species is compared with one or more multicellular species. This approach can inform us about the presence/absence of genes and expansion/contraction of gene families in multicellular species compared to the unicellular ones. The limitation with this approach is that it is difficult to disentangle lineage-specific changes from those that are associated with the evolution of multicellularity. One remedy to this problem is to use independent instances of the evolution of multicellularity and use comparative phylogenetic methods to infer the common genetic changes related to the evolution of multicellularity (Cornwallis & Griffin, 2024).

Comparing two genomes of multicellular and unicellular organisms and recovering homologous sequences often reveals that the orthologous protein coding sequences are diverged from one another. A fundamental question is which of the observed substitutions are drivers of a particular evolutionary change and which ones do not contribute to the phenotype of interest. A possible solution to this would be to use tests of positive selection. Positions that show a signature of selection in multicellular lineages might have led to a specific change, for example, addition of a phosphorylation site to a downstream kinase target. Identifying targets of positive selection can therefore allow us to distinguish driver substitutions related to the evolution of interdependent multicellular networks.

Finally, the diverse array of tools used in molecular cell biology can help us test evolutionary hypotheses in organisms amenable to molecular manipulation. In the case of evolution of cell cycle control, we have a wealth of knowledge from decades of research in this field which we can use to understand the design principles of adding new inputs to existing cell cycle machinery. One possible way is to identify pairs of unicellular and multicellular species in lineages which are amenable to experimental studies and focus on putting together the genetic circuitry required for intercellular dependence of cell cycle control piece by piece. Such an example can be found in the green algae where multicellularity has evolved multiple times independently. The green algae (Chlorophyta) present a unique system. Most studies have been focused on the Volvocine algae, however, other branches, Ulvophyceae present very useful systems (Umen & Herron, 2021). To fully understand the evolution of molecular mechanisms of interdependent cell cycles, it is crucial to simultaneously use both evolutionary genomics methods together with cell and molecular biology.

From the perspective of interdependent cellular networks defined in this work, multicellularity is not defined simply by the presence of many cells surrounded by an extracellular matrix, but by the stable restriction of cellular autonomy over survival and reproduction, enabling these processes to be regulated collectively across the organism.

Table 1. Guiding questions to study the evolution of cellular interdependency in the transition to multicellularity.

	Question to ask
1. Life cycle	When is the organism unicellular vs. multicellular? How long the organism stays in each stage?
2. Cellular autonomy	Can a single cell survive on its own? Can a single cell reproduce on its own?
3. Survival interdependency	Under what conditions, cell require other cells to survive?
4. Division control	What controls cell division? Is decision to divide reliant only nutrients or also signals from other cells?
5. Reproductive unit	Which cell (s) are responsible for reproduction?
6. Plasticity	Can cells change their functional role in the organism depending on signals from the environment?
7. Cellular memory	How stable are cellular identities? What mechanisms are responsible for cellular identity?
8. Dependency network hierarchy	Are all cells equally critical for the survival and reproduction of the organism? If not, how is the hierarchical cellular network defined?

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