AGENCY IN THE EVOLUTIONARY TRANSITION TO MULTICELLULARITY

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ABSTRACT

This review explores *agency*, behavior intrinsic to an organism and initiated by it, as it relates to the development of multicellular organisms and its evolution. We ask how agential behaviors contribute to and change concomitantly with evolutionary transitions from unicellularity to multicellularity, including the evolution of animals from their closest unicellular antecedents. We consider the relation of agency to the organization and autonomy of multicellular organisms and conclude, surprisingly, that it is not as strict as it is for individual cells. The main reasons are previously unacknowledged morphogenetic inherencies of multicellular matter and developmental capacities to amplify and partition functionalities of constituent cells. These modalities generate novel phenotypic enablements that enhance the scope of agential behavior. We discuss experimental approaches to distinguish between agency and evolved, program-like behaviors of organisms, including purposeful actions. We argue that evolved complexities of animal development make it unsuitable for exploring experimentally single-cell-to-multicellular transformations in agency and focus our attention instead on agency in the life cycles of social bacteria and amoebae, and in the transitions between multicellular and unicellular states in cancer. Finally, we discuss mathematical representations of incompletely specified dynamical systems and how they may be used to characterize biological autonomy and agency.

INTRODUCTION

Although it is difficult to consider the concept of agency in the abstract, it is relatively easy to name features whose possession immediately suggests agency. Behaviors that appear to be goal-directed and indicate a sense of purpose are two of them. Such features have long been taken as characteristics of living, but not non-living, entities. Since agency appears to inhere in all free-living systems, it is a likely attribute of single-celled organisms and at least some cells derived from multicellular entities (Baluška and Reber, 2019; Lyon et al., 2021; Baluška et al., 2024; Rosslenbroich et al., 2024).

While sometimes dismissed as a legacy of vitalist thinking, agency has become newly prominent as a component of evolutionary theory with the rise of interest in niche construction and evidence for organism-initiated activities in inhabiting their environments (Odling-Smee et al., 2003; Walsh, 2015): in Lewontin's framing, the organism as the subject, not simply the object, of evolution (Lewontin, 1983).

By way of examples of agential features, life forms seek resources from their environment to promote their survival. Typically, they do this by moving, reorienting their position, or sending out physical extensions of themselves. These activities are based on internal drives and motives that are *dispositional*; that is, they can change (e.g., selection of nutrients, or with respect to the rate and directionality of their movement or growth) when the environment changes (Anjum and Mumford, 2018a,b) (See below and the Glossary).

This review seeks to explore how single-cell agency may have been integrated into multicellular developmental processes during the evolution of animals, plants, and other complex organisms, and how the agency of the developed organism reflects its single-cell origins, but also can be transformed into something qualitatively new. We discuss physical determinants of mesoscale materials in these transformations insofar as they have been shown to play causal roles in multicellular form, as well as the operation of gene regulatory modalities in the origination of novel cell types and multicellular functions. Our approach is to emphasize identifying the right questions more than providing definitive answers. A glossary is included in order to make the meaning of certain terms clear to the general reader.

Some scientists and philosophers take an instrumentalist approach and equate the statement that an entity possesses agency to the inability of an external observer to fully account for a

change in state based on what is known, or can reasonably be inferred, regarding three potential determinants of the change: (a) its internal dynamics; (b) the stimuli that impinge on it; and (c) how those stimuli are transduced to changes in internal variables and eventually to the new state. According to this view, if the entity appears to "have a mind of its own" or "act on its own behalf" (Kauffman, 2000), it may simply be that we lack the tools to specify the determinants of its actions.

The view that agency is just apparent, a placeholder for that which we do not presently understand in strictly causal or deterministic terms (Sapolsky, 2023), is one pole of a range of views relating to degrees of self-motivated action in any organism and what is experienced as free will in conscious ones. The opposite pole is belief in willed action that is not completely constrained by antecedent physical determinants, with counterparts of this down to the cellular level; that is, genuine agency (Mitchell, 2023). A variety of positions in between are termed "compatibilism" (McKenna and Coates, 2024).[1] For the purposes of this review, we entertain the possibility that agency is a real factor in living systems and their evolution rather than just a symptom of our ignorance as observers.

The following is a list of what we see as the most important questions related to agency. However, because we wish to restrict our attention to transitions in the levels of agency from single cells to multicellular organisms, we will *not* address the first two of them in any detail (the remaining five are italicized for emphasis).

(i) What is the range of entities that have agency? Is it just organisms, i.e., all forms of single-cell and multicellular life, or does agency also pertain to derivatives of cell- and organism- based systems, either natural or human-fashioned, e.g., viruses, computers, robots? Alternatively, is agency even more primitive than cellular life, pertaining to the chemical systems that preceded and engendered life? Further, can non-living material or immaterial systems exhibit agency (Goff, 2019)?

(ii) When did agency emerge in the history of the cosmos, or of the Earth? If (in answer to point (i)), cells are considered the ground state of agential matter, an upper limit could be

[1] One version of this is to heuristically treat organisms as intentional agents, even though they might not be, under the assumption that natural selection provides rational solutions to challenges. See discussion in Okasha (2024) The Concept of Agent in Biology: Motivations and Meanings. *Biological Theory*, 19:6-10.

placed by paleomicrobiology. But if chemical systems can have agency, was there a major transition in this respect that was prior to, or coincident with, the origin of life? At what stage can individual cell behavior be thought of as agent-like?

(iii) How does the faculty of agency relate to other capabilities and functions of living systems? Does it make sense to distinguish between an organism's (a cell or multicellular entity) being an agent from its manifesting agency? That is, is an agent's agency always "on"?

(iv) How was agency aligned in cell collectives and integrated into multicellular entities, and exported to novel forms, in the evolutionary transition from unicellular to multicellular organisms? What cellular changes (if any) were needed to bring this about?

(v) Can one draw useful parallels between the evolutionary shift of cell agency from single cells to multicellular groups, the analogous developmental shift during the life cycle of myxobacteria and dictyostelid amoebae, and the formation and dissolution of cell groups in cancer?

(vi) What experimental procedures allow us to define and measure observable indicators of agential behavior at different scales (e.g., cells, cellular aggregates, motile pseudoplasmodia)? Can agential properties change while other properties (e.g., genetic, biochemical, etc.) do not? To what extent does agency depend on prior causal determination? Are there aspects that conflict with prior determinants? Are there experimentally observed behaviors of cells as they enter and leave multicellular assemblages which elude explanation after all apparently relevant parameters have been considered?

(vii) How can mathematical and computational modeling help us understand agential systems? Do such models need to be incompletely specified, given the possibility that no currently characterized physical processes or standard mathematical representations can capture all the "degrees of freedom" (a mathematical term with possible biological implications) of an agential system?

Questions *(iii)-(vii)* therefore direct our focus on the extent to which agency has been a missing or unrecognized factor in explanatory narratives and models of organismal development and the evolution of development (i.e., evolutionary developmental biology; Love, 2024; Müller, 2021; Walsh, 2015) and how agency of multicellular organisms may differ from that of single cells.

There are constitutively multicellular prokaryotes (Kumar et al., 2010) and ones that are transiently multicellular (the myxobacteria, discussed below, are examples). Although the earliest unambiguous multicellular eukaryote fossils date from 1.63 billion years ago, the earliest accepted unicellular eukaryotes are from the same deposits and of similar age (Miao et al., 2024). It is therefore unclear how much time it took for multicellularity to first evolve, and how or if the cells' agency might have promoted it. Subsquently, depending on the criteria applied (e.g., cell–cell attachment, cell communication, division of cell labor) eukaryote multicellularity evolved anywhere between 10 and 25 independent occasions (reviewed in Niklas and Newman, 2013; 2020). The heterotrophic eukaryotes, among which the holozoan progenitors of the animals emerged, are thought to be no older the 800 million years (Sebé-Pedrós et al., 2017). Multicellular organisms can be classified either as aggregative ("coming together") or zygotic ("staying together"), according to the mechanism by which multicellularity arises (Bonner, 1993; Grosberg and Strathmann, 2007).

While the motivating interest of this review is to understand the evolution and development of animal multicellularity from its closest unicellular ("nonmetazoan holozoan") ancestors to its reversals and reconstitutions in cancer, we mainly draw on evidence and examples from other (i.e., noneukaryotic and nonholozoan) lineages, such as social bacteria and amoebae, in speculations on metazoan origination scenarios. The reason for this is two-fold. First, animal embryos are products of more than 600 million years of evolution from the time the metazoans split off from the nonmetazoan holozoans. While their constituent cells exhibit strong evidence of agential behaviors (see below) every stage of their natural lives is intrinsically social, and none are conducted as unicellular agents. Second (and correspondingly) when multicellularity is achieved by the coming together of separate cells (as it is in the life cycles of social organisms), it offers an ideal opportunity to track agency at the two levels. How do its manifestations differ as the "individual" changes from a single cell to a group of cells? Similar reasoning motivates our exploration of the coming together and disassociation of cancer cells.

Finally, the questions discussed here involve a multiplicity of perspectives and disciplinary discourses. Inevitably, there are differences of opinion, some of them major (even among the present authors, though all endorse the six conclusions in the Discussion). Most relate to the key question of agency, whether "an animal [i.e., a cell or multicellular organism] itself, rather than merely events and states going on in its parts, might be able to bring something about."[2] While we provide a balance of views, we also point out significant disagreements that await resolution or reconciliation. To emphasize the polyphonic nature of this paper we have organized the main text as a series of themes. The eight numbered Themes map onto our key questions (iii)-(vii) above in the following manner: questions *(iii)* and *(iv)*, which cut across many issues and concepts of organismal identity, development and evolution are considered under Theme 1 (Agency at the Cellular and Organismal Levels), Theme 2 (Biological Manifestations of Agency), and Theme 5 (Reshaping of Form, Repurposing of Function, and the Emergence of New Enablements). The examples of social microorganisms and cancers (question *(v)*, above) are discussed, respectively, in Themes 3 and 6, and questions *(vi*) and *(vii)* are covered in Theme 7 (Experimental Challenges in the Characterization of Agency and its Transformations and Theme 8 (Mathematical and Computational Modeling of Putatively Agential Systems).

THEME 1: AGENCY AT THE CELLULAR AND ORGANISMAL LEVELS

There have been multiple, often conflicting, proposed characterizations of agency (e.g., Barandiaran et al., 2009; Kauffman and Clayton, 2006; Mitchell, 2023; Moss, 2024; Okasha, 2024; Pickering, 2024; Rosslenbroich et al., 2024; Sultan et al., 2022; Virenque and Mossio, 2024; Watson, 2024; Woodford, 2019), we will assume for our analysis that a biological framing of agency consists of five independent properties:

(a) the ability of the entity ("self") to continuously (though sometimes transiently and provisionally) demarcate itself from its environment ("non-self") and actively constitute and reconstitute its boundary (Plessner and Bernstein, 2019).

(b) the "drive" of the system to maintain and repair itself and flourish over time.

[2] This is a phrase used in the publisher's description of Steward, H. (2012) *A metaphysics for freedom*. Oxford: Oxford University Press, but not in the book itself. We thank Rani Lill Anjum for bringing it to our attention.

(c) the capacity of the system to explore and react to significant features of its environment, and to adapt in response to external perturbations, potentially by modifying them or by changing itself internally.

(d) the capacity to enter into relations with other agents with a myriad of possible consequences, including their mutual bootstrapping into a qualitatively different level of agency.

(e) the capacity to engage in self-initiated idiosyncratic, possibly hedonic, or potentially self-destructive activities, that is, activities with no pre-established connection to species-characteristic survivability, adaptation, or sociability.

We view these abilities, drives, and capacities dispositionally, that is, as propensities (*sensu* Popper (1959)) or inherencies (Newman, 2017) that may or may not manifest themselves. This means that a particular response to an environmental situation of an agent may tend to go in a certain direction but need not do so (Anjum and Mumford, 2018a,b). In other words, an agential system has the capacity to respond to the same situation in more than one way (or will appear to, to an observer with limited information (see below), and this may in fact be one of its necessary conditions. We consider the possibility that the responses comprised by this dispositionality are not due merely to inherent stochasticity but rather due to what is implied by agency – prerogatives of the system that, at least at present, defy any purely deterministic characterization.

A collective of cells could potentially exhibit forms of agency qualitatively different from that of its constituent cells if it constitutes a new form of matter, i.e., it exhibits emergence (Anjum and Mumford, 2018b).[3] The multicellular entity may be demarcated from the external environment by relatively persistent cell-cell associations based on attachment proteins or extracellular materials (e.g., "slime" in social bacteria or amoebae), accompanied by a particulate to liquid-like phase transition. Sustained proximity is thus an evolutionary starting point of a novel multicellular form. Analogous phenomena occur in the genesis of sociality in insects and even humans. The basis of the binding together of individuals will be different in each case, and is just the prerequisite for, not the defining character, of what might be, or become, a new form of matter. If this occurs it will be accompanied by novel inherencies.

[3] The emergence of novel forms of matter from existing ones is familiar in cases like the atoms of the chemical elements forming from plasma as the universe cooled, or liquid water condensing from gaseous water molecules.

A relevant question is whether the collection is a transient (though possibly recurrent) entity (as with biofilms and some social bacteria and amoebae), a new kind of individual, or something in-between. The emergence of a novel, multicellular, form of individuality with altered agential properties might be a gradual process based on its becoming a Darwinian unit of selection, and thus following a divergent evolutionary trajectory (a standard supposition in evolutionary theory). But a collection of cells also might become a novel agent relatively abruptly, as a consequence of becoming a new form of living matter with novel properties (see Newman (2019) and (Newman, 2020b)).

Survival strategies for multicellular entities include cooperation based on intercellular exchanges of chemicals and mechanical signals, or on a division of labor whereby different coexistent tissues in the body perform complementary functions via differentiated cells and organs. Cell assemblages that are integrated individuals can evolve with respect to their forms and functions. Natural selection is based on different variants leaving different numbers of offspring in successive generations. The usual assumption (which may be violated; see De Monte and Rainey, 2014) for such fitness-based evolutionary processes to operate, however, is that the organismal entities are genetically uniform. But some multicellular forms (e.g., bryozoans) can be genetically chimeric or otherwise different from their originating population (e.g., transgressive hybrids in land plants). In such cases other persistence strategies are adopted – such as a partial physiological coupling among tissue domains (Shapiro, 1996) or the colonization of novel ecological niches – out of competition with their relatives (Rieseberg et al., 2003), and therefore independent of relative fitness.

THEME 2: BIOLOGICAL MANIFESTATIONS OF AGENCY

In our conception, agency is a faculty of all living systems, unicellular and multicellular. Under the reasonable assumption that cells ancestral to present-day metazoan animals exhibited agency, what role might agential acts have played in their lives? In what way could it have been different from other life-sustaining properties?

Agential behaviors may be among the functional attributes, even indispensable ones, of a living system (Garson, 2019). However, every functional activity need not be a manifestation of agency. For example, navigation of a maze by the plasmodial slime mold Physarum (Reid, 2023) or pursuit of a mating partner by a sparrow could be characterized as activities that are both

functional, since they contribute to the individuals' or species survival, and agential, because they are in some sense optional, and involve decisions or choices. The initiation of cell division when the amoeba reaches a certain mass, or the formation of segments in a bird embryo, are also functional in the above sense, but are evolved, automatic, behaviors over which the cell or multicellular individual exerts no choice. It would therefore not be useful to count them as indications of agency. This point is especially relevant when agential acts involve "spur-of-the-moment" responses to stimuli. Such responses may have functional roles, and the capacity to perform them may have evolved, but not the acts themselves. In contrast, the interests of living systems are often served by stereotypic responses to internal or external cues, responses which are functional, but not agential.

To distinguish further, some apparently goal-directed activities, rather than being agential, are physically inevitable, analogous to a ball rolling down an inclined plane. In cells, for example, the uptake of small essential molecules can occur passively, by transmembrane diffusion along chemical gradients. In the early-stage embryos of some animals, the sorting out and partitioning of differentially adhesive cells are thermodynamically driven (once the suitable components are in place) (Steinberg, 2007), and therefore inescapable. Such processes have been termed "teleomatic" by Mayr (Mayr, 1988).

Other goal-directed processes – those termed "teleonomic" (Mayr, 1988; Pittendrigh, 1958) – do not have this inevitable character, but occur because they have evolved to operate in a particular manner. While they fully conform to the rules of physics and chemistry, their organizational contexts and operation out of thermodynamic equilibrium produce unusual outcomes. Uptake of nutrients by cells *against* external gradients, DNA synthesis, muscle contraction, and embryonic development are examples. They are sometimes referred to as machine-like, but this characterization has been disputed (Nicholson, 2019). Teleonomic processes contribute to organismal survival by virtue of their highly reliable outcomes.

Many theorists believe that most developmental and behavioral processes are deterministic in the teleonomic sense (though with increasing rejection of the notion of literal "genetic programs"; Nijhout, 1990; Moczek, 2012). The implication is that, except for a narrow range of variability due to inherent stochasticity, these processes are machine- or computer-like, with in-principle predictable outcomes, even if we do not have enough information to describe the programs. This leaves little or no scope for agency, as described above. Here we provisionally

pursue the view that agency in sense of organism-initiated action that defies strict determinism (as argued by Steward, 2012 and Anjum and Mumford, 2018b; see also Mitchell, 2023) does, in fact, exist. (How this can be consistent with a naturalistic metaphysics is discussed as part of Theme 8, below.) Since individual organisms are different from one another in distinctive ways, not all their inclinations necessarily follow their species's evolved behavioral patterns. This quirkiness may have random aspects, but it can also be idiosyncratically causal, that is, subject to internal processes that are not characteristic of members of the organism's species. It may sometimes make them more successful than their cohorts, and sometimes less so. The suggestion is that authentic agency comes with the propensity for choices that have not been shaped by selection for reproductive success, at least not to begin with.

Further, an organism, be it unicellular or multicellular, can initiate activities inimical to its own well-being. A cell might, for example, navigate toward an attractant that ultimately turns out to be poisonous. Such behavior might depend on an evolved program, but with the unforeseen outcome of a deceptive (to the organism) external stimulus. In multicellular entities, moreover, continued existence, survival strategies, and agency pertain not to individual cells but to the organism as a whole. Therefore, individual cells can be enlisted to undergo behaviors that are disadvantageous to their own survival or even fatal, but advantageous to the multicellular organism. Apoptosis – developmentally regulated cell death – is the canonical example of this, and a classic case of the transfer of agency from a single cell to a group of cells.

The idea of agency opens the door to a naturalized understanding of drives that reflect the propensity of living forms toward maintaining themselves or seeking satisfaction. They may use myriad means to do so. Each of these means, taken by itself, need not obviously favor maintenance, and the proclivity for even risky or fallible explorations may accompany agency. Agential activities, even if their main role is to promote survival, do not need to do so in every instance so long as they do so on average.

THEME 3: FROM UNICELLULAR TO MULTICELLULAR AGENCY

A transition from the unicellular to the multicellular state could merely involve a shift in the unit that manifests agency. Multicellular agency may be analogous to an intensive physical property like the viscosity of a liquid, which depends on the specific interaction between its particles (e.g., cells, see below) or a colligative property like the freezing point depression of a

solution, which scales with the number of particles (e.g., cells, see ref. (Nanjundiah and Sathe, 2011)). On one side of the shift there is a set of individual cells that appear to behave like independent agents. On the other side, the same set behaves like a single agent, a collective. The shift may or may not exhibit a steep dependence on the number of constituents, i.e., in terms of agency, the transition may occur smoothly or abruptly. Also, the transition may be "noisy": the unit of agency may be poorly defined near the boundary of the transition. Sufficiently far from the dividing line, the new unit is markedly different in size, number, and identity of constituents or behavior relative to the old unit. The transition may take place by purely short-term physical interactions, but it is characterized by a set of novel features that further the integrity and functioning of the whole. (See Arias Del Angel et al., 2020 for a detailed consideration of the relationships among physical and agential effects the transition between individual and collective cell behaviors,)

However, what if we do not restrict ourselves to an instrumentalist view, and attribute objective reality – not just descriptive utility – to agency at the cellular level? If we attribute objective reality, and not just descriptive utility, to agency at the cellular level, is multicellular agency still a straightforward consequence of cell numbers or physical interactions, or is it something different? In population genetic terms, a multicellular entity would constitute a new unit of natural selection which can contribute to reproductive fitness via heritable traits expressed at the collective level. Multilevel selection theory, e.g., Buss (1987), Falk and Sarkar (1992), Folse and Roughgarden (2010). holds that the long-term persistence of multicellular individuals depends on the playing out of natural selection acting on heritable variation concurrently at the group, multicellular, unicellular and genetic levels (Lewontin, 1970). Viewed thus, on one side of unicellular-multicellular divide, there is a potential for conflict between individual fitness and collective fitness. The question arises, if agential capacities are intensified by complexity and differentiation, would new forms of agency provide the multicellular state with an intrinsic advantage in this conflict of interests?

Transiently multicellular forms like social bacteria or amoeba present a different challenge to the conflict model of multilevel selection theory. Here, what is interpreted as fitness-maximizing group behavior may be better viewed as the outcome of many individual behaviors being simultaneously brought into alignment by agential behavior rather than "genetic interest" (Lewontin, 1970). In metazoans, where multicellular activity that is potentially agential is a

factor in most, if not all, life cycles, it may emerge as a spontaneous (i.e., emergent, unprecedented) consequence of being composed of agential cells, but on a different spatial scale and with interactional constraints among the cells. More generally, if a novel organismal form exhibited modes of agency that its direct antecedents did not, it might seek and inhabit a different ecological niche from the members of its originating population and their more typical progeny, one suitable to the flourishing of the collective. Classical measures of fitness based on numbers of offspring per individual in a population of organisms competing for common resources would be inapplicable in such cases.

The placozoan *Trichoplax adhaerens*, an early-emerging animal, shows that novel agential capacities can arise in a multicellular grouping without, so it would seem, any of its individual members having evolved new adaptations. Rather than specialized tissues, appendages, and organs, Trichoplax conducts its life as a multicellular entity by coordinating the physical properties of the most basic cell-derived properties. This permits us to discern what may be a primordial form of novel agential capacities. For example, ultrafast contractions of the dorsal epithelium (not underlain by a stiff basement membrane as in more complex animals; Armon et al., 2018) enable Trichoplax to move and capture prey. In addition, the cilia of the placozoan's ventral surface (direct unicellular derivatives) undergo a concerted behavior which appears to be based on a similar physical principle to that underlying the flocking of birds (Bull et al., 2021). (See the more detailed discussion in Newman, 2023b). The novel features of animals that are conventionally thought to endow them with agential capacities (e.g., nervous systems and brains) are conceived as elaborate products of evolution (i.e., "adaptations"). But these placozoan capabilities appear to emerge by physics-based "self-organization"[4] of preexisting cellular functionalities, something that is more abruptly achieved.

The appropriation or alignment of unicellular agency to produce agential multicellular forms can have occurred in several ways. If the ancestral cells had intrinsic sociability, e.g., a propensity to communicate or benefit from resources they could provide one another, the origination of the multicellular entity could have been mutualistic. This could be a gradual process of increasing interaction between single-celled organisms accompanied by the emergence of a consensus or higher order set of norms. But it might also have occurred as an

[4] Energy-consuming phenomena that lead to concerted motions and nonuniform arrangements in material systems, See below, and Theme 4.

automatic effect of new surface proteins or matrix molecules that were sticky or entrapping, or preexisting ones that acquired these properties with environmental changes. (Metazoan cadherins function as adhesive molecules only when ambient Ca^{2+} is sufficiently high, for example (Halbleib and Nelson, 2006).) When cell-cell associations result from non-elective physical scaffolding rather than social interactions, one might speak of cells losing some of their individual agency.

New material properties spontaneously emerge in aggregated, or more generally, collective systems. Viscosity in liquids, mentioned above, is one such property, as are the new states of matter brought about by phase transitions. An example of the latter is the transition from an (ideal) gas, where particles effectively do not interact and intermolecular forces are negligible, to the liquid state, where particles interact continuously with their neighbors via intermolecular forces. This is typically associated with an abrupt change in the degrees of freedom of the system's subunits. We can thus speak of the same subunits, in different contexts, constituting distinct forms of matter, with different inherent properties.

Differences in agency in multicellular vs. unicellular organisms might derive in part from their being distinct forms of matter. Gas-like to liquid-like changes in state of a multicellular entity occur as swarms of cells (as in the social amoeba *Dictyostelium discoideum*) become streams and mobile multicellular pseudoplasmodia, or "slugs" (Fig. 1). Although the cellular subunits bear the hallmarks of agency, their transition from individual to collective motion can partly be explained by the physics of phase transitions pertaining to nonliving systems (Arias Del Angel et al., 2020). The reverse physical transformation in tissues, loosely analogous to a liquid-to-gas transition, is termed "epithelial– mesenchymal transformation" (EMT). Here a cohesive tissue becomes a collection of separate cells (Amack, 2021). EMT occurs during animal embryogenesis, for instance, when cells detach from the neural tube at the embryo's central axis and migrate to distant sites, forming peripheral nerves and other tissues.

Agency has also been attributed to multicellular plants, particularly the land plants (embryophytes), where it has been asserted to be essential to their behavior (Baluška and Mancuso, 2021; Gilroy and Trewavas, 2023). These organisms consist of a unique, solid form of biological matter, in which the cells are immobile, but are in intimate and long-range communication via physical channels – plasmodesmata – and diffusible molecules (auxins) (reviewed in Niklas and Newman, 2020).

Because their subunits are living cells, the novel forms of matter represented by multicellular aggregates are liable to be "excitable media" (Levine and Ben-Jacob, 2004), "active matter" (Bernheim-Groswasser et al., 2018; Gross et al., 2017)), or both.[5] They will thus have properties not readily predicted by physical laws formulated for conventional viscoelastic materials. Being physically inescapable, such dynamical supracellular attributes do not constitute forms of agency by themselves. But they are potential "enablements" (see below) that the resulting multicellular agents could employ in new ways of life.

Since cellular slime molds have life cycles with both unicellular and multicellular phases, they can provide examples of transitions between different levels of agency, scaffolded by the respective physical states. When the apparently agential amoebae cease their exploratory activity and converge into liquid-like streams, their mode of transport is no longer primarily individual motility, but rather bulk flow, a "generic" effect that also pertains to nonliving systems (Newman and Comper, 1990). When the streams organize into collectively motile slugs, however, the mode of transport partly reverts to a dependence on individual, potentially agent-type effects. While physical forces still help propel the cell collective forward (Rieu and Delanoe-Ayari, 2012), subpopulations of cells in the slug are differentially responsive to distinct external signaling molecules, and rearrange accordingly (Feit et al., 2007). After a fruiting body forms and the motile slug no longer exists, a subpopulation of cells, the spores, physically detaches from the apex, eventually to develop into the freer agents represented by the original amoebae. (A similar reinstitution of less constrained agency also occurs in the life cycles of prokaryotic myxobacteria; reviewed in Arias Del Angel et al., 2020). Spores are simultaneously end-states of the developmental process and precursors to the amoebae (or motile bacteria) and not themselves migratory. They are thus a state of differentiation. and possibly of agency, distinct from those of cells at other developmental stages.

One manifestation of agency is exploratory behavior that is underdetermined by externalities. (Once again, this could reflect limited knowledge of the observer and would become fully determinate once all intracellular variables are also specified, but alternatively could represent genuine organism-initiated decisions.) Cells operating within developmental

[5] Excitable media are materials that expend stored energy to propagate signals (e.g., chemical, electrical, mechanical), potentially repetitively, but with a refractory period between events. Active matter is a class of materials consisting of subunits that expend energy to move, or to exert mechanical forces.

systems like the life cycles of social amoebae or animal embryos are clearly curtailed with respect to this exploratory capacity, but this may be a facultative (i.e., conditional) rather than constitutive (i.e., evolved/fixed) loss.

Cells isolated from embryos are clearly agential, capable of biologically relevant behaviors under experimental culture conditions, e.g., navigating through mazes toward nutrients (Tweedy et al., 2020). In situ, however, they typically locomote randomly, confined in a liquid-like state by reversible adhesive interactions. Thus, while in multicellular settings animal cells do not manifest agency under all circumstances, they are nonetheless agents by nature. (This differs from the suggestion that living organisms, when acting non-agentially, depart from this status (Barandiaran et al., 2009).) We can infer from this that cell agency can be subordinated in a collective, and that such subordination can persist through the evolutionary duration of a multicellular lineage, but that it is not necessarily permanent, e.g., genetically inscribed. This suggests that any relinquishment of aspects of unicellular agency may be to some extent cooperative, and thus itself elective and agential.

While developing embryos present difficulties for discerning unalloyed organismal agency, the cell aggregates constructed from prospective ectoderm of the Xenopus blastula ("biobots"), studied by Levin and his colleagues (Blackiston et al., 2021; Kriegman et al., 2021), represent an informative simplification of such systems. These submillimeter spheroids consisting of several thousand cells can navigate mazes and engage in a novel behavior in which free cells are gathered by the fabricated cell aggregates into new clusters. The activities appear to employ multicellular agency, but of a type that the constituent cells did not evolve to perform.

Scenarios for the origination of multicellular agency will typically postulate that preexisting unicellular organisms undergo modifications in their agential capabilities as they become integrated into novel forms. This is difficult to theorize for the life cycles and developmental programs of extant organisms whose lineages continued to evolve for hundreds of millions of years after multicellularity was initiated. Agential qualities at different levels of organization have not only coevolved with one another and the physical effects that scaffold them but have come to serve new evolutionarily acquired organism-level purposes (Newman, 2023a)

THEME 4: AGENCY IN RELATION TO PURPOSIVENESS AND AUTONOMY

In exploring the relationships between the agency of single-cell and multicellular entities and possible differences in the nature or manifestations of agency in these forms, it is necessary for us to also consider the agency-enabling properties these entities share. The notion of agency is part of a long history of attempts to characterize the essential, distinctive properties of living systems. A well-known proposal by Immanuel Kant is that organisms are "natural purposes," defined as entities that are both the causes and effects of themselves (Kant, 1790; trans. 1966). By this definition, no such thing is found in the nonliving world. Tornadoes, for example, are centers of recruitment to the concerted motion of air and sometimes earth and water. Physicists refer to such phenomena as "self-organization." Although Kant himself coined this term to describe natural purposes, tornadoes do not produce the materials they comprise, so they clearly do not satisfy Kant's criteria for living organisms. Physical self-organization (including the reaction–diffusion pattern-forming instabilities and other "dissipative structures"[6] described by Turing (Turing, 1952) and Prigogine and coworkers (Goldbeter, 2018; Prigogine, 1980)) contributes to the dynamics of living systems, and likely to their origins, but is different from biological self-organization (Moss and Newman, 2015; Newman, 2022a; 2023a).

Though Kant doubted it was amenable to scientific analysis, empirically informed philosophical approaches to what Kant identified as intrinsic purposiveness were framed in terms of organization and nonequilibrium thermodynamics by the early to mid-20th century organicists, a collective of theoretical biologists that included Woodger, Needham, Waddington, and Bertalanffy (reviewed in Gilbert and Sarkar, 2000; Haraway, 1976; Nicholson and Gawne, 2015). Of particular significance is the notion of *autonomy*, discussed by Russell as early as 1930 (Russell, 1930). The concept has been defined more recently (with Kant's criterion foregrounded) as a characteristic of "organized systems, which are able to self-produce and self-maintain as integrated entities, to establish their own goals and norms, and to promote the conditions of their existence through their interactions with the environment" (Moreno and Mossio, 2015a). An influential, albeit abstract, proposal for how this is accomplished is Maturana and Varela's *autopoiesis*, which they characterized as pertaining to a "machine [sic] organized…as a network of processes of production (transformation and destruction) of components" (Maturana and Varela, 1980; p. 78). The *organizational approach* of Moreno and

[6] Spatially and/or temporally (e.g., oscillatory) phenomena that arise in thermodynamically open systems due to consumption of energy.

Mossio and their coworkers is an important extension of autopoiesis that incorporates the concept of the constrained thermodynamic work cycle, which Kauffman considers an intrinsic and necessary aspect of living systems (Kauffman, 2000). The "organization" of the organizational approach is "closure of constraints," in which mutually supportive work cycles construct one another's constraining conditions (Moreno and Mossio, 2015b, see also Bechtel and Bich, 2024).

The 19th and 20th centuries saw the recognition of the cell as the unit of life. Autopoiesis and the organizational approach, as modern theories of autonomy, were originally based on physically and chemically plausible, but abstract, organizational principles of this fundamental living unit.[7] While each has been extended to include more complex living entities such as multicellular animals and plants, ecosystems, social formations, and so forth, the applicability of the basic principles of operational or organizational closure are less clear when it comes to supracellular entities. The notion of autonomy in both the Kantian natural purpose sense and the modern senses requires organismal "selves," or individuals (Varela, 1991) (although the definition of the latter is often controversial (Wilson and Barker, 2024)). Transient colonies of social bacteria and amoebae (discussed above) are not individuals as conventionally understood, although their constituent cells at some life-cycle stages fit the description. Further, the existence of eusocial organisms like bees and naked mole rats, and (with respect to the evolution and use of language, for example) humans (Deacon, 1997), shows that the concepts of individuality and autonomy are not absolutes.

Agency is not the same thing as autonomy, though the two go hand-in-hand. It is difficult to conceive of an agential entity (i.e., one capable of interacting purposefully and nonautomatically with its environment) that is not at least partly autonomous (i.e., self-contained and -generating), and an individual could not be autonomous in a given setting without exhibiting some form of agency.[8] As the examples above show, both agency and autonomy of cells in a collective can

[7] We do not intend to suggest that the autonomy of single-celled organisms is absolute in any sense and acknowledge the philosophical discourse around the constitution of the identity of all organisms in important part by the physical and biotic environments in which they are embedded (Dengsø, M. J., and M. D. Kirchhoff. 2023. Beyond Individual-Centred 4E Cognition: Systems Biology and Sympoiesis. *Constructivist Foundations*, 18:351-364, Walsh, D. M. 2015. *Organisms, agency, and evolution*. Cambridge: Cambridge University Press.) The significant points for us here are that unicellular life arose before multicellular life and that the former, in all examples with which we are familiar, is internally organized in a coherent fashion in a way that the latter only is in certain cases.

[8] Kant also had a concept of agency, but it was tied to his notions of morality and rationality, and therefore not implicit in his idea of natural purpose Korsgaard, C. M. 2008. *The constitution of agency : essays on practical*

differ at different stages of its life cycle or developmental trajectory. Regarding the subject of this paper – agency in the evolutionary transition to multicellularity – it is important to recognize that the phyletic antecedents of the animals – the nonmetazoan holozoans (e.g., choanoflagellates, ichthyosporeans; Sebé-Pedrós et al., 2017) – have extant forms with transient and facultative multicellular morphotypes (Larson et al., 2020; Sogabe et al., 2019; Tikhonenkov et al., 2020), but none of these has been found to exhibit phenotypic and behavioral plasticity comparable to those of even the simplest metazoans. The evolution of animal agency must have taken different routes than those represented by these forms.

Working within the organizational approach, Arnellos and Moreno distinguish a "constitutive" and an "interactive" component of multicellular entities and state that "agency is only possible in such systems if there is "a radical entanglement between the related processes," which they term "the constitutive–interactive closure principle" (Arnellos and Moreno, 2015, p. 333). They suggest that this principle in turn requires a "regulatory system functionally integrating the two dimensions [i.e., a nervous system] and…a special type of organization between the cells [i.e., an epithelium]" (Arnellos and Moreno, 2015, p. 333). Multicellular agency in this view would only pertain to eumetazoans, not the early diverging "basal" metazoans, sponges and placozoans, which lack both nervous systems and epithelia. It would certainly not apply to the biobots in the experiments described above (Blackiston et al., 2021; Kriegman et al., 2021).

Without getting into too-detailed a comparison of the described perspective with conclusions presented by us here and previously (Newman, 2023b), we suggest that the organizational approach features an unduly stringent notion of functional integration for qualifying multicellular entities as agents. It has long been recognized that non-animal multicellular forms such as the Dictyostelium slug can exhibit apparent agency (Bonner, 1994). Regarding the animals, the primitively specialized cell types of placozoans are not integrated into anything resembling organs (Newman, 2023b; Smith et al., 2014), and in locomoting and digesting bacterial prey these animals depend on cytological features no more elaborate than those found in ancestral cells (discussion above, in Theme 3).

Further, however, the autonomy and agency of even fully integral animals are not necessarily tied to genetic uniformity or species identity. Experimentally constructed embryo

reason and moral psychology. Oxford ; New York: Oxford University Press.

chimeras formed from the blastomeres of sheep and goats (estimated to have diverged 14-16 Mya), resulting in "geeps" (Fehilly et al., 1984), or from medaka and zebrafish (teleost lineages that separated on the order of 320 Mya; Hong et al., 2012), are viable and healthy. But they have body and organ phenotypes that are intermediates, or compromises, between those of the originating species.

In the transition from unicellular to multicellular organisms, some phenomena not captured by standard evolutionary theory or models of cellular autonomy may have been in play. If, for example, morphological or functional novelties can arise by nonadaptive means (see the discussions of Trichoplax and biobots, above), there is no requirement for them to participate in preexisting systems of closed constraints. As described in the next section, organismal agency can be viewed as driving, rather than reflecting, evolution at the multicellular level.

THEME 5: RESHAPING OF FORM, REPURPOSING OF FUNCTION, AND THE EMERGENCE OF NEW ENABLEMENTS

In an agency-centered view of evolution (i.e., the organism as its subject, not as its passive object; Lewontin, 1983; Walsh, 2015), traits are usefully considered *enablements* rather than *adaptations*. Adaptations in the standard picture are evolved traits that improve the ability of organisms to meet challenges of existing or changing environments relative to populational cohorts. If they are genetically underpinned, they need to arise gradually, since large deviations from the phenotypic norm would be unlikely to perform better than features which had evolved in previous cycles of competition (Fisher, 1930). Different shaped bird beaks suited to consuming different seeds are classic adaptations.

Enablements, by contrast, are features that could be novelties initially lacking specific functions, but later, when their bearers use them to forge new ways of life, become essential to the survival of the lineage and its individual members. Body segments of animals across multiple phyla, the antennae of insects, and the paired appendages of vertebrates, are examples of enablements. They can be incidental outcomes ("spandrels") of developmental processes (Gould and Lewontin, 1979) or appear suddenly due to mutations ("hopeful monsters" *sensu* Goldschmidt, 1940) or as side-effects of the readjustment of such processes (Müller, 1990). If they are not deleterious, they can persist (Bonner, 2013), and the organisms in which they appear

will be free to invent things to do with them (Munoz-Gomez et al., 2021; Stoltzfus, 1999; West-Eberhard, 2003).

As Kauffman has noted (using the screwdriver as a stand-in for all features that appear with no precedent or evident function in evolutionary lineages), "no rule-following procedure, or algorithm, can list all the uses of a screwdriver; and…no algorithm can list the next new use of the screwdriver" (Kauffman, 2019 p. 119; see also (Longo et al., 2012). In the adaptationist framework these features have been referred to as preadaptations or exaptations. Here we consider them instead as enablements, launching their bearers (since they are agential) into ecological domains in which their comparative fitness to their progenitors is irrelevant to their persistence.

Enablements can be *morphological* or *functional* (and often both). The evolution of new morphological enablements occurs by effects that reshape and topologically reconfigure tissue masses during development (Forgacs and Newman, 2005; Newman and Comper, 1990). This can occur even without the differentiation of new cell types, simply by the rearrangement of existing cells (Salazar-Ciudad et al., 2003). If such effects result from genetic alterations, they will be heritable. If they are induced by environmental effects they can continue to be expressed so long as they are not detrimental, and may eventually, due to mutation, become genetically assimilated into the developmental program (Crispo, 2007; Waddington, 1957).

As noted above, the association of cells during development or evolution, due to extracellular matrix materials in social bacteria or amoebae, or cell surface adhesion molecules in the metazoans and their presumed ancestors, generates novel forms of excitable or active matter, each with characteristic inherencies. In the animals, particularly the eumetazoans (all except the placozoans and sponges) sets of "dynamical patterning modules" (defined as gene products of the conserved "developmental toolkit" (Carroll, 2005) and the physical forces and effects they mobilize (Newman and Bhat, 2009)), elicit latent morphological propensities of the multicellular materials, leading to the formation of layers, interior cavities, segments, appendages, and external and internal skeletons (Newman, 2012). While not themselves constituents of agency, these motifs provide the resulting organisms with new ways of interacting with their environments.

In contrast to morphological innovation, the evolution of new functional enablements typically involves the emergence of new cell types and organs. Unlike physical reshaping,

functional differentiation has no nonliving counterpart. It is a key phenomenon of and causal factor in the transition from unicellular to multicellular agency (see Newman, 2023b).

As noted above, the morphologically uncomplex placozoans survive and flourish with fewer than a dozen primitive cell types and no appendages or organs (Smith et al., 2019; Smith et al., 2014). Further, the "biobot" experiments indicate that a cluster of undifferentiated embryonic cells, by employing unicellular functionalities in new ways, can survive by means unrelated to any functions of the tissue from which they were derived, or even the organism of origin (Blackiston et al., 2021). This implies that cell differentiation and organogenesis may not be essential to the ability of multicellular entities to fashion modes of agency that differ from those of their constituent cells. Why then did such entities evolve? One proposal is that specialized cell types with ready-made functions were consequential to a chromatin-based "differentiation engine" that appeared coincidently with the animals (or all but the placozoans) (reviewed in Newman, 2020a; Newman, 2023b). This apparatus appears to have been poised to mobilize preexisting complexes of co-regulated genes and to amplify their expression in subsets of cells. Once it was in place, a panoply of cell types could evolve by a relatively small number of steps.

The nonmetazoan holozoans had all the *functionalities* (nutrient uptake, metabolism, biosynthesis, motility, detoxification, excretion of wastes, sensation, and a few others) required to live. Moreover (as noted above), just being composed of such cells is apparently sufficient for a multicellular entity to persist. This suggests that the body plan embellishments of complex organisms represented by specialized cells, tissues, and organs are in principle nonobligatory (an animal can live without one or more limbs, muscles, or eyes, for example), or at least were so at their inception.

The possession of morphological and functional "add-ons", however, can enable new ways of being (cf. von Uexküll's *Umwelten*: organism-specific ways of experiencing and inhabiting the environment; Feiten, 2022), and may therefore contribute to new forms of agency (Newman, 2023b). These novelties, initially optional, but ultimately lineage-defining and generatively entrenched (Wimsatt and Schank, 2004), may not have arisen by incremental selection to meet environmental challenges (i.e., as adaptations). Rather, they could have appeared by more abrupt mobilization, amplification, and partitioning of intrinsic cellular functionalities that created new modes of exploration. Employing and extending a sensory-theoretical notion of Gibson's

(Gibson, 1966), Walsh has asserted that agential beings evolve by using phenotypic novelties to invent new "affordances" (Walsh, 2015).

The appropriation of essential cell functionalities to produce initially inessential but agency enhancing capabilities in multicellular organisms entails a general but underrecognized evolutionary phenomenon of establishing the independence of an aspect of an integrated whole. This has been termed "detachment" (Moss, 2006). Detachment can lead to new enablements if it is followed by repurposing (variously termed "subfunctionalization" or "neofunctionalization;" Voordeckers et al., 2015). This combination of effects constitutes a different category of transformation from the self-organizational and other reshaping processes that underlay the evolution of morphology and was a major driving force of the phylogenetic increase of functional complexity (Newman, 2019, 2020a).

The partial separation and repurposing of an aspect of an integrated whole can leave the original capabilities in place but enhanced. The reproductive budding of the invertebrate Hydra is an example of this (Bode, 2009). Another is the cell differentiation process in all metazoans, which are enhanced in their capabilities by the presence of fewer than ten (in placozoans) or up to 200 or more cell types with intensified functionalities. From the standpoint of the differentiated cell, however, subfunctionalization, both evolutionary and developmental, typically leads to its being deficient in relation to ancestral capabilities and the capacity for independent existence. Specifically, such cells may lose the ability to divide (e.g., skeletal myoblasts, neurons), locomote (e.g., hepatocytes, chondrocytes), undergo oxidative phosphorylation (e.g., brown adipocytes), or all three (erythrocytes). Thus, cells give up aspects of both their autonomy and agency in the multicellular metazoan setting.

Deficit-creating forms of detachment and subfunctionalization can induce a drive toward further differentiation (Moss, 2014). For the differentiated cells, further differentiation (e.g., of digestive or motile cells) will be needed to support and integrate useful ones into the organism's body and behavior. Some of these compensatory functions (e.g., a vascular system for delivering processed nutrients to interior loci) would have been general-purpose. They would have enabled further detachments of single-cell functionalities as components of new kinds of tissues and organs. Such compartmentalization would also have facilitated functionally modular phenotypic variation (Gerhart and Kirschner, 1997).

In summary, in the absence of specialized cells or organs, preexisting single-cell functionalities (e.g., ciliary activity, contractility, secretion) can be recruited in the service of unprecedented forms of multicellular agency (exemplified in the biobot experiments).[9] This suggests that one manifestation of a life-form's (unicellular or multicellular) agency is the capacity of to be creative. Agency is not identical to the condition of being alive (an erythrocyte has this property but does not seem to exhibit agency[10]), but neither is it something brought into existence by embellishments or additions (an epithelium, a nervous system). If functional or morphological "add-ons" do become available (as they clearly have throughout evolution), they would have represented novel enablements for exploring previously inaccessible ecological niches and for identifying new environmental affordances (Gibson, 1966).

Correspondingly, the evolution of development can be characterized as being accompanied by an upward transition of the locus of function from individual cells to tissues and organs, or in the case of cellular slime molds, from amoeba to slug. This entailed a loss of homogeneity via functional differentiation of the constituent cells. Along with morphological innovation (based on physical reorganization of of tissue masses), complex body plans emerged through anatomical compartmentalization (tissue layering, segmentation, appendage formation, and so on) and intensification of capabilities.

As higher-order forms and functions arise, the independent agency of cells is traded off against a greater independence of the collective. The capacity of individual cells to participate in such a trade-off may itself be an expression of cellular agency, and outcome representing the emergence of a new "normative field" (Moss, 2021).

THEME 6: TRANSFORMATIONS OF MULTICELLULAR AGENCY IN CANCER

An implication of the described processes for generation of multicellular agents is that the higher-order entities constitute new kinds of biological matter with novel dispositions and inherencies (Newman, 2019; 2023a; Newman and Niklas, 2018). With such "downward

[9] In items (i) and (ii) in the Introduction, we set aside the questions of whether derivatives of living systems (e.g., proteins, organelles, and other "smart materials") can exhibit agency, and whether chemical systems that preceded life can be considered proto-agential. These are clearly separate questions, and the foregoing does little to resolve them.

[10] In item *(iii)* in the Introduction we asked whether it made sense to distinguish between an organism's being an agent from its manifesting agency. While it is not obvious that an erythrocyte or other entirely dependent cell type is an organism, it is clearly alive. Our analysis leads us to the conclusion that while being agential is inseparable from being alive, the opposite is not necessarily the case.

causation" between-group variation can be less than within group variation, e.g., "sister" slugs, or two members of an animal species, may be more similar than cells within those multicellular forms. The propensity of cells, multicellular tissues/organs, organisms, etc., to realize a "normativity," i.e., a higher-level ordering or set of emergent inherencies that supervene over internal heterogeneity, may be a defining condition of natural agency (Moss, 2021).

In the opposite causal direction, neoplasia or cancer begins when the ability of a tissue to override the individuality of its heterogeneous constituent cells lapses. As that happens, rather than continuing to achieve agential integration at the tissue level, a cell (or group of cells) begins to reestablish its individuating agency and detaches it from the tissue-level regime (Moss, 2021; Soto and Sonnenschein, 2021).

Neoplasia tends to "progress" in a stepwise fashion, suggesting that downward agential transitions are incremental, and that tumors at various stages represent *sui generis* multicellular systems. For example, the extracellular matrices of tumors can induce novel cell heterogeneities distinct from those characteristic of development (Pally et al., 2022). These, in turn, acquire novel morphological enablements through physical self-organization (see Theme 4) (Langthasa et al., 2021; Pramanik et al., 2021) and, in principle, multicellular agency. It may therefore be a misconception to view cancer as mere antisocial activity by individual cells escaping from the tissue or organ-level collective. It may be more productively thought of as a succession of novel multicellular forms (literally "neoplasms") constituting new normative regimes establishing affordances provided by the host organism (Germain and Laplane, 2017; Lean and Plutynski, 2016).

Reasoning the other way around, the pathological reversals of cancer may provide insight into multicellular origins. What we know regarding the transformations and reorganizations from tissues to tumors suggest that the upward transitions involved in the evolution of organismal agency may have also involved incremental and reciprocal cascades. The exploratory properties of the multicellular forms in each case, for instance, could differ from those of individual cells in part because of new enablements accompanying cell differentiation. The loss of differentiation in tumors can lead to high-risk (for the cancer cell population as a whole) exploration, while neo-differentiation of sub-lineages generate new enablements suitable to new (e.g., organ-specific) niches (Pradeu et al., 2023). These features are all manifested in ovarian cancer (Fig. 2), which

has become an experimental paradigm for pathology-enabling multicellular-to-unicellular transformations and reversals in morphology and apparent agency (see Theme 7).

THEME 7: EXPERIMENTAL CHALLENGES IN THE CHARACTERIZATION OF AGENCY AND ITS TRANSFORMATIONS

Although we have not defined agency beyond its being potentially unprogrammed cell- or organism-initiated behavior, we have discussed some of its properties, manifestations, and transformations. Empirical and experimental approaches are crucial for the validation, enrichment, and expansion of this inevitably largely philosophical discourse. For example, experiments can potentially identify constraining and scaffolding determinants acquired in the transition to multicellularity. Such factors can measurably limit the range of activities of individual cells, and thus the ambit of their agency, but simultaneously produce entities with new enablements and agential capabilities. Like the constraining and scaffolding effects, new enablements (particularly morphological novelties) will often result from the mobilization at the multicellular scale of previously unavailable physical processes, including self-organizational ones (Newman, 2019; Newman and Bhat, 2008).

The new functions that arise in the evolution of multicellular forms are typically amplified and partitioned (i.e., into specialized cell types and organs) counterparts of the life-sustaining functionalities of their unicellular antecedents (Newman, 2023a,b). While signatures of the expression of agency at the single-cell or multicellular levels of organization, or in the transit between them, can be elusive, they can show up (as mentioned above) in experiments in which cells (Lyon et al., 2021; Tweedy et al., 2020) or multicellular aggregates (Blackiston et al., 2021; Kriegman et al., 2021) are placed in settings different from any conceivably encountered during their evolutionary history.

Experimentally investigating agency, including the conditions of its operation in the context of transitions between unicellular to multicellular states in developmental and evolutionary time, entails several challenges. One of these is the selection appropriate biological models and to delineate their advantages and limitations. For instance, slime molds and myxobacteria are good models for studying the development of multicellularity by aggregation (e.g., Arias Del Angel et al., 2020; Nanjundiah, 2016; Ramos et al., 2021), while yeasts and cyanobacteria can be used to

study the transition to multicellularity in clonal or "staying together" scenarios (e.g., Baselga-Cervera et al., 2023; Ratcliff et al., 2012).

These model organisms have also provided – and continue to provide – key insights into the evolutionary transition to multicellularity, mainly because of likely similarities between their contemporary biophysical context and that in which multicellularity may have first arisen, such as the spatial and temporal scales and low-Reynolds number[11] media in which they live. Further, the existence of extant genetically related but phenotypically divergent species suggests the character of the morphospaces in which these organisms evolved, and thus some of the constraints on the varieties of their agential properties (see references in Arias Del Angel et al., 2020). The volvocine algae provide paradigmatic examples of these features (de Maleprade et al., 2020; Umen, 2020). In utilizing these systems, however, it is important to avoid interpretations that appeal to "living fossils" or "ladder [of progress] thinking" (Omland et al., 2008).

A second challenge has to do with the nature of the environment and organism-environment interactions in the study of agency and agentive behavior. Most biological models have been selected, at least in part, because of their amenability for laboratory studies, and are cultured in tightly controlled or constant conditions. Though this is a reasonable and fruitful approach, the study of development and evolution in changing and ecologically relevant environments has not developed as fast as other lines of research, such as the impact of genetic changes in constant environments (Bolker, 2014). The biases entailed by these limitations present technical, methodological, and analytical challenges that are being addressed by a variety of strategies (Rivera-Yoshida et al., 2018).

A related issue is how to formally incorporate other cells as part of the studied cells' environment. Even in relatively low-density populations, the motility and behavior of individual cells can be modified by cellular density, cellular contact, and by external determinants that are impossible to classify as only cell-cell or cell-environment interactions. Indeed, during the developmental transition to multicellularity in social bacteria and amoebae, and in tumorigenesis, cells create their own microenvironments that reflect back on and modify the physico-chemical

[11] The Reynolds number is defined as the ratio of the inertial forces to the viscous forces of a fluid Purcell, E. M. 1977. Life at low Reynolds number. *Am. J. Phys.*, 45:3-11.

processes that produce them (e.g., Huber and O'Day, 2017; Langthasa et al., 2021; Ramos et al., 2021).

A third challenge in studying the transition to multicellularity with an agency-informed perspective is defining the object or target of study, which often turns out to be dynamic and fuzzy. During aggregative development of the social bacterium *Myxococcus xanthus*, for example, cells may move and "act" as single individuals, as small clusters, as streams, or as more complex 3D structures, depending, e.g., on cellular density, substrate properties, cellular age, or other factors (Rivera-Yoshida et al., 2018; Thutupalli et al., 2015). Therefore, even for the same model organism and even under the same initial conditions, the experimental challenges in recording the manifestations of agency can and will most likely change as multicellular development proceeds.

Prokaryotic social bacteria, however tractable experimentally, can only provide a small window into the roles of agency in the development and evolution of multicellularity. Comparative experimental studies of eukaryotic organisms with life cycles containing both unicellular and multicellular phases, such as the Dictyostelid group of social amoebae (the cellular slime molds: CSMs; Bonner, 2009), can take us further, including testing inferences regarding metazoans, which, as mentioned, present difficulties in identifying agential determinants due to the coevolved complexities of their developmental systems Fig. 1).
Here we start with two hypotheses. First, a multicellular stage evolved from free-living unicellular ancestors. Circumstantial evidence in favor of this is strong, even compelling (Bonner, 1967, 2009; Sebé-Pedrós et al., 2017; Nanjundiah et al. 2018). Second, in common with manifestations of the living state generally, agency is exhibited in both stages. The overwhelming majority of work on the CSMs, however, has concentrated on a single species, *D. discoideum ("*Dd*").* Since Dd is an example of an "advanced" – meaning relatively recently evolved – species (Schaap et al., 2006; Sheikh et al., 2018; Swanson et al., 2002), studies on it are as likely to tell us about trait accretions that followed long after the transition as about those that facilitated it.

The ambiguities inherent to this model have been reinforced by the convenience of handling Dd in the laboratory. This has meant that even within the species, most attention has been directed at one or the other of a handful of mutants for growth under axenic conditions, i.e., free from other organisms. The mutants ((Watts and Ashworth, 1970);

http://dictybase.org/strain_history.htm) develop "normally" but whether they are pleiotropic regarding subtle molecular details that may have been significant for the unicellular–multicellular transition is unknown. Since laboratory strains differ significantly regarding developmental details, considering robustness of the broad features of the life cycle (Nanjundiah, 2019), this possibility cannot be ignored.

Plasticity within species can mimic characteristic features of other species. Multicellular morphologies show back-and-forth phylogenetic transitions (Romeralo et al., 2013) and highlight an important point not restricted to the Dictyostelids or amoebozoans. Namely, ideas of what is simple (= "ancestral") and what is complex (= "evolved"), which are primarily based on morphology, bear no relation to DNA-based phylogenetic assignments of ancestral and derived states. In other terms, "*grades* of organizational complexity need not reflect *clades* of closest relatives" (Nanjundiah et al., 2018). Because aggregative multicellularity has evolved independently at least five times in the six eukaryotic supergroups (or eight times depending on how one counts; Tice and Brown, 2022), common mechanisms behind the transfer of agency are more likely to be ascertained from comparative studies than by examining a single species. Unfortunately, we lack sufficient details about development in members of the other eukaryotic supergroups to decipher any commonalities in single-cell properties that may lie behind unicellular-to-multicellular transitions.

We are thus restricted to experiments on *D. discoideum* primarily, and *D. giganteum* and one or two other CSMs, secondarily. Two questions need to be addressed: Are there functional or agential traits possessed by the multicellular state but not by single cells? If so, what are they?

The differences we seek between the two states are qualitative, not quantitative. An example of the latter is the efficiency of movement of a cell collective, which depends on the balance between the motive force generated by each unit and the drag caused by friction with the substrate: the larger a mass of cells, the faster it can move. But what appears to be a qualitative difference may not be one after all. The ability to form a fruiting body with a spore and a stalk would appear to demand multicellularity. However, a single amoeba of *Protostelium,* also an amoebozoan, can secrete an extracellular stalk, ascend to the top, and encyst itself to form a spore. The fruiting body bears an uncanny resemblance to a Dictyostelid fruiting body (Olive and Stoianovitch, 1960).

Because persistence of cell–cell attachment is the defining condition of multicellularity, a change in the system of cell-cell adhesion is the likeliest candidate for a determining, or at least a scaffolding, role in shaping a transfer of agency during the unicellular to multicellular evolutionary transition. In line with our assertion above that the acquisition of classical cadherins served this role in the transition to metazoan multicellularity in the holozoans, we might compare the CSMs and unicellular amoebozoans for the appearance of a new gene or genes associated with the transition in this clade. However, both for the reasons discussed above and because of the failure of such an approach in other systems (e.g., the Volvocine algae, in which the single celled Chlamydomonas and the multicellular Volvox possess what are practically identical gene sets; Prochnik et al., 2010), we must allow for the possibility that the change may have been quantitative (e.g., via DNA sequence amplification) or a subtle alteration in gene regulatory profiles.

It might be more useful to look for indirect evidence. That can be carried out as part of the theoretical and modeling work described separately in this review, which does not make any assumptions regarding any molecular basis for the transition. Comparative analysis will play a central role in this. We know that a wide range of morphologies pertaining to aggregation, cell type distributions within the aggregate (slug), and the fruiting body exists between different CSM species, both "on the average" and as exceptional variants within the same species (Bonner, 1967, 2009; Nanjundiah, 2016; Romeralo et al., 2013). It should be possible to introduce schemes of cell-cell adhesion into models that are based on the mechanical and chemotactic behavior properties of single motile cells. Parameter variation can then be performed to see if any of the models exhibits an appropriate range of alternative developmental morphologies.

The CSMs thus afford the possibility of experimentally defining a range of molecular, behavioral, and physical determinants in the unicellular-to-multicellular transition that go beyond mere reproductive fitness considerations to organism-initiated factors. The opportunity to perform comparative and multiscale studies and to intervene at precise stages of the organisms' life cycles are highly advantageous in pursuing these elusive effects.

Whereas (as noted above) cells in the embryos of extant metazoans are typically scaffolded by mesoscale physical effects and do not behave as independent agents during development (Newman, 2013), cancer, represents a pathology of animal biology that arguably provides more

insight into the origination and evolution of such forms than their present-day ontogenetic processes (Davies and Lineweaver, 2011). As with myxobacteria and CSMs, migratory cancer cells move through diverse microenvironments that exert distinct mechanical and biochemical influences on them. The metastasis of cancer across body cavities or coeloms has long been thought to occur through the formation of spheroidal clusters of disseminated single cells. This has led researchers to use experimental models that are dependent on the adhesion of suspended cancer cells. There is, however, mounting evidence that spheroids may form in vivo through the dissemination of already aggregated cells (Micek et al., 2023). The ordering of the events determines the clonal diversity within each spheroid, and hence, its capacity for survival and metastasis.

It is unsurprising therefore that diverse multicellular modes of migration are adopted by motile tumor populations, each perhaps with their own agential aspects (Friedl and Wolf, 2003). An intriguing example of morphological heterogeneity is seen in disseminating ovarian cancer. In this condition, the peritoneal fluid tends to harbor distinct multicellular spheroidal morphotypes: grape-like dysmorphic clusters that are structurally labile, and more a resilient lumen-containing phenotype (Brown et al., 2023; Langthasa et al., 2021) (Fig, 2).

Defining and characterizing the relevant behavioral units of such heterogeneous phenotypic manifestations in developmental and oncological systems, and devising experimental, imaging, and analytical tools to rigorously study them, are the main challenges for these studies. This requires working with increasingly complex models such as laboratory strains of mice. Such *in vivo* systems are often intractable to real-time microscopic examinations and are fraught with inconsistencies between anatomical and physiological features of rodents and humans. Assays based on organoids, tumoroids, and tumor-on chip systems incorporate biophysical and histological complexities of human tumor microenvironments, while at the same time allowing high throughput measurements of parameters associated with progression and treatment (Ingber, 2022). It is important, however, not to conflate the model systems with natural ones in attempts to detect agential activities, where the distinctions may be more important than for other behaviors.

Given the multiplicity of phenotypes and behavioral modes seen in progressing cancers, we can ask at what point agency per se, as distinct from other developmental (e.g., morphogenetic, differentiative) effects, might be exerted during tumorigenesis? A recent study provides some

suggestions (Attalla et al., 2023). A critical determinant of the therapeutic response in tumors has been characterized in terms of "hot" and "cold" tumor–immune microenvironments (TIME). The former is associated with infiltration by T-cell and other immune cells and anti-tumor cytokine production, with low proliferation, invasiveness, and metastasis, with the latter having the opposite properties (Wang et al., 2023). Attalla and coworkers showed that the tumor cells influenced the character of their immune environment by differential post-transcriptional use of certain mRNAs (Attalla et al., 2023). If a role for a tumor's agency in advancing its own fate indeed exists, it might be found at the level of such subtle effects.

THEME 8: MATHEMATICAL AND COMPUTATIONAL MODELING OF PUTATIVELY AGENTIAL SYSTEMS

Biological agency may elude strict determinism or rule-governance. How can this be described mathematically? If cells or organisms embody evolved programs capable of being characterized mathematically or computationally modeled such that all behavioral eventualities are specified regardless of externalities, it would be difficult to attribute true agency (as discussed above) to the respective entities. Physico-chemical causation of organismal choices or behaviors cannot be rejected in its entirety, but as described above, it can be dispositional rather than absolute. We therefore seek to characterize formal representations that are *underdetermined* in the sense that the system's causes, whether external or internal, derive in part from factors complementary to the model.

Developmental processes involving continuous media (e.g., viscoelastic or compressible tissues, diffusible morphogens) changing over time and space are typically modeled using differential or integrodifferential equations (Murray, 2002). For each set of initial conditions, these systems generally have unique (deterministic) solutions. To mimic the noisiness of real systems, deterministic factors can be combined with stochastic ones in the framework of stochastic differential equations, making solutions probabilistic (Øksendal, 2007).

In contrast, when the behavior of cell collectives is modeled, an individual- or "agent-based" discrete approach is a frequently used strategy. In a biological context it is useful to consider spatial models with nonuniform geometry in which the individuals are identified with motile cells. Here rules (deterministic or stochastic) are assigned to each model cell, which may be the same for all, or differ between subpopulations. The cells are permitted to perambulate randomly,

subject to executing internally specified actions when they encounter another cell, or a feature of the environment designated to elicit a programmed response.

Some developmental models incorporate both discrete and continuum modes, when, e.g., slime mold amoebae aggregate and enter streams (Maree and Hogeweg, 2002). Slime mold aggregation can also be modelled as the consequence of a physical instability that sets in beyond a critical spatial density (Keller and Segel, 1970). This model can reproduce several characteristic features of *D. discoideum* aggregation, including the formation of inwardly streaming cells (Nanjundiah, 1973), but it is not clear whether such novel behaviors accompanying the evolutionary emergence of multicellular collectives represent novel forms of agency or "only" exotic material properties (see Theme 7).

Our discussion earlier in this review suggests that biological agency is something more than strict determinism plus stochastic effects. How might mathematical models of development and its evolution be modified or reconfigured to introduce features like idiosyncratic motives accompanied by species-atypical, nonadaptive, or even (from the viewpoint of survival of the individual or the group to which it belongs) reckless behaviors?

One relatively straightforward way of accomplishing this, using individual-based models, is to introduce individuality in the sets of internal rules. The goal would be to make some of these versions of the species-typical ones that are likely to have resulted from successive cycles of survival-driven natural selection ("move up a gradient of attractant," "attach to another cell on contact"), but others in (also deterministic) defiance of these rules. To what extent can such motivational and behavioral outliers be tolerated and carried along by the collective (i.e., not expelled as "cheaters"), potentially providing genetic repositories for meeting future external change, or just adding a lifelike anarchic aspect to the resulting multicellular entities? (See De Monte and Rainey, 2014, for a model of a way this could be realized.)

Continuum models, which are typically based on physical principles or laws (unlike the arbitrary rule books in the cells of individual-based models), represent more of a challenge for simulating the uncertainty and idiosyncrasy of organismal activity not solely attributable to randomness. This is because they are by necessity coarse-grained descriptions in which the behavior of individual cells is replaced by the spatiotemporal development of averaged cell densities, making it difficult to account for individual differences.

There is, in fact, a formal body of mathematics suitable to representing continuum processes in a world organized in this way that leaves room for factors such as biological agency. Pattee has framed the question of potential alternative pathways in biological systems not in terms of determinism plus randomness, but as a structural property of the dynamics of such systems: "[T]here must be more degrees of freedom available for the description of the total system than for following its actual motion…Such constraints are called *non-holonomic*" (Pattee, 1971).

In contrast to the holonomic (integrable) systems typically encountered in physics and chemistry, the potential outcomes consistent with these nonholonomic constraints are path-dependent, and none can be excluded a priori. The addition of equations of motions that respect the nonholonomic constraints, however, can render such systems deterministic in the sense that each initial condition specifies a unique solution (Bloch et al., 2005; Flannery, 2005).

In mathematical terms, nonholonomic constraints characterize dynamical systems in which the number of degrees of freedom needed for the description of the system overall is greater than the effective number of degrees of freedom for the actual motion at each point in time and space.[12] That is, there are fewer a priori inaccessible regions of state space. Such systems can be represented by inexact differential (or Pfaffian) forms, i.e.,

$$\sum_{i=1,\ldots,n} f_i(x_1, \ldots, x_n)\, dx_i = 0 \qquad (1)$$

Physically, this means that the extent and direction of possible changes in the state variables $(x_1, \ldots, x_n)$ are constrained. If the constraint is integrable, i.e., it can be written in the form $dh = 0$ for some function $h(x_1, \ldots, x_n)$, it is thereby holonomic and the inaccessible regions of state space increase, i.e., fewer variables are needed to describe its state.

In systems or subsystems in which there is insufficient information to derive a complete set of dynamical equations, inexact differential representations can be useful in a practical sense. Examples include chemical reaction networks (where the state space is defined by the range of possible concentrations of each chemical), and idealizations of ecological and other complex networks. They are typically expressed as ordinary differential equations of the form,

[12] See Hooker, C. 2013. On the import of constraints in complex dynamical systems. *Foundations of Science*, 18:757-780. for a detailed consideration of nonholonomic constraints in biodynamical systems.

$$\frac{d\vec{x}(t)}{dt} = f(\vec{x}, t, P) \quad (2)$$

where $\vec{x}$ is the multidimensional system state, which changes with time according to the function f. The unique values of f are determined by that state, subject to parameters P. For incompletely specified systems, however, the topology and directions of influence of the components of could still be described via differential forms (Delphenich, 2012; Newman, 1970). Such nonholonomic systems could denote relationships among variables that are causally related, but not strictly so, thus also providing a natural representation of intrinsically dispositional systems as described, e.g., by Anjum and Mumford (2018a,b) (see discussion under Themes 1 and 2).

This approach would replace a set of differential equations governing the temporal development of the system with a less deterministic framework, constraining the set of possibilities of temporal development without committing to unique deterministic or even stochastic trajectories. This can be mathematically framed in terms of the concept of differential inclusions (Smirnov, 2002), i.e., generalizations of systems of differential equations where the right-hand side of (2) is instead replaced by a *set* of possible derivatives, $\frac{d\vec{x}(t)}{dt} \in F(\vec{x}, t, P)$. The idea of using differential inclusions to mathematically characterize the actions of living systems is advanced in Aubin's Viability Theory (Aubin et al., 2011), but its applications to basic biological processes have thus far been mainly abstract.

While this is not the venue for elaborating a mathematical description of biological systems embodying dispositional causation, we suggest that this could be a fruitful approach to the agency question. Positing that determinism of the conventional microstate -> microstate type is incomplete enables a role for downwardly causal determinants, including subject-initiated guidance of its own fate.

Finally, reflecting the incomplete determinism that characterizes any single level of causality in a biological system, it is reasonable to anticipate that "multi-method" frameworks will contribute to the understanding of some of the questions discussed here. For example, the Glazier-Graner-Hogeweg model (Cickovski et al., 2005; Hirashima et al., 2017) brings together discrete, individual-based and continuum approaches for the interplay of dynamics at different levels of organization. Although its notable successes have been in

the modeling of morphogenesis in multicellular embryos (Adhyapok et al., 2021; Belmonte et al., 2016; Chaturvedi et al., 2005), applications to the life cycle of cellular slime molds (Marée and Hogeweg, 2001), multicellular bacteria (Ramos et al., 2021), and tumorigenesis (Pally et al., 2019) demonstrate its relevance to phenomena where agency may play a more prominent role.

DISCUSSION

Agency joins an increasing number of topics – purpose, intrinsic evolutionary directionality, cognitive qualia, free will – that have the unusual status of compelling the interest of some scientists and philosophers while having their very existence questioned by others, or even most. In this paper we examined evidence for the agential properties of cellular life (while resisting the temptation to develop a full-fledged theory of agency), and we have asked how such agency is alternately recruited, integrated, released, and reinstituted during the life cycles and development of social microorganisms and the formation of cancers.

The cases we consider are deliberately transient and reversible. While we speculate on the inferred transition in the holozoan clade that led to the metazoan animals, given the more than half-billion years of evolutionary history since the origination of the latter group we do not focus on changes in the manifestation of agency in the development of extant animal species.

Our default assumption is that living systems are dynamical entities that change from moment to moment in a manner that depends on their present constitution, history, and external forces. It might follow from this view that the extent and direction of change would be guided by recognized principles of physics and chemistry. Even in this conventional formulation, however, the description of life on earth and its evolution would elude strict deterministic description in terms of the changing physico-chemical composition of living matter.

The best-known limitations to full determinism in biological systems are unforeseeable spontaneous effects intrinsic to them. These include mutations (germline and somatic) to genetic systems and incidental epigenetic changes to nucleic acids. Also included in this category are the nonlinearities of physiological and developmental dynamics that introduce the random selection of alternative trajectories (bifurcations) in systems starting from the same initial state.

Because the determinants of living systems extend beyond the above-mentioned “recognized principles of physics and chemistry,” there are sources of uncertainty other than randomness.

The materials represented by multicellular aggregates (variously "excitable media," Levine and Ben-Jacob, 2004 and "active matter," Bernheim-Groswasser et al., 2018; Gross et al., 2017, see footnote 2) have properties that are not readily predicted by physical laws formulated for conventional viscoelastic materials. In addition, many cellular proteins, particularly those involved in signaling and gene expression, show varying extents of intrinsic disorder (Uversky, 2015) (defying Anfinsen's famous principle of sequence-structure determinism) and constitute, along with non-protein-coding nucleic acids, "biomolecular condensates" materials that are partly glass-like and partly gel-like, with capacities (unlike those of aqueous chemical systems) to store and render information. There are no recognized physical or chemical models of the structure-function relationships in these materials (Swain and Weber, 2020).

Further, the internal organization of cells is of central importance to life. The heterogenous lipid, protein, polysaccharide, and nucleic acid assemblages constituting all prokaryotic and eukaryotic cells are, as far as understood, necessary conditions for their functional activities, including putative agential ones. Although formal principles of this cellular organization (Maturana and Varela, 1980; Moreno and Mossio, 2015b) and quasi-chemical models for its realization (Deacon and García-Valdecasas, 2023; Hofmeyr, 2021) have been advanced, its minimal physico-chemical bases and how they emerged from the nonliving world remain enigmatic. Theories of multicellular agency that build on unexplained cell-level agency must be counted as "methodological vitalism" (Walsh, 2018). This includes those that derive from autopoiesis and the organizational approach, but also, to the extent that we refer to cell-level phenomena, the synthesis presented in this paper.

In contrast to the origin of single-cell functionalities, there are plausible first-principle scenarios (once the properties of cells are assumed) for the emergence of the anatomical and functional traits of multicellular forms. We have briefly reviewed evidence for evolutionary and developmental transformations of physical state based on inherencies of relevant materials, including liquid-tissue formation, liquid-liquid phase separation, solidification and so forth, producing novel morphological motifs in social bacteria and amoebae, animal embryos, and tumors.

The functional elaborations of multicellular organisms, such as motile pseudoplasmodia or tissues and organs, are based on the stage-dependent institution and deployment of specialized cells during the life cycle. We have described the process, in metazoans, of developmental

amplification and partitioning of intrinsic cell functionalities. Like the evolution of morphological complexity, functional complexity is based on inherencies (Newman, 2017), but ones that already evolved in the ancestral cells. We framed the evolution of cell differentiation as an example of detachment and subfunctionalization, general phenomena that have proved applicable as well to the reversals and reconstitutions of multicellular organization seen in cancer.

Unlike the gradually produced innovations posited by population-based models, which are inevitably adapted to the environments in which they evolve, the novelties discussed here – which draw on often saltational transitions between inherent morphological motifs or repurposing and partitioning of preexisting cell functionalities –persist only in relation to the agency of the organisms that carry them. They are "Kauffman's screwdrivers" (Kauffman, 2019), capable of creating novel affordances and thereby defining new forms of life. In this sense, agency begets new forms of agency.

The experimental strategies we describe for identifying potentially agential behaviors and transitions between them are cognizant of the fact that not all exploratory or goal-directed activities are agential. Apparent chemotaxis of cells on a 2D plane, for example, can be the result of random motion that is speeded up in the presence of increasing concentrations of a chemical substance, i.e., chemokinesis (Zigmond et al., 2001). Experiments that place cells or cell aggregates in situations that would not have been encountered in the evolution of their lineage – such as confronting myxobacteria with artificially textured surfaces or cancer cells with aberrant matrices (or biobots with mazes) – and seeing whether they "invent" new modes of behavior, are ways of detecting evidence of agency.

Even if the novel behaviors are not fitness-increasing in the populational sense, or even obviously promoting of survival, if they are consistently responsive to the novel challenges and adaptive in conceivably realistic situations they may count as genuine creative activities. It should be acknowledged, however, that no finite set of such experiments would convince a committed determinist that the appearance of individual willfulness is authentic agency rather than being predestined by the prior state of the universe or dictated for each organism by its evolutionarily endowed teleonomic program (Sapolsky, 2023).

An alternative to a categorical determinism where some events or situations are strictly determinative of others, is *dispositional causality*. In this framework, causal processes are

"powers" or inherencies that may cause things to happen, but not inevitably. Anjum and Mumford contend that most activities exhibit dispositionality and that pure contingency or necessity, while not impossible, are generally untenable abstractions. The context-dependence of causation is captured in their assertion that "[a] causal process will begin once a disposition meets its appropriate partner(s) and starts interacting. During this process some properties will be lost, and new properties and new interactions might be introduced"(Anjum and Mumford, 2018b; p. 80). They propose that "science should be about uncovering the real causal powers of things as evidenced in their tendencies" (Anjum and Mumford, 2018b), p. 138).

Among the dispositional factors relevant to agency, autonomy was discussed in Theme 4. Autonomy is a fundamental property of cells, though it can be relinquished by them, as seen in the examples of social bacteria and cellular slime molds. *Intentionality*, another such faculty, the property of being directed toward some object or situation, contains the implication of deliberate choice, an ability of "minded creatures" (Anjum and Mumford, 2018b, p. 152). While the attribution of mindedness is beyond the scope of this review, there is evidence that it pertains in some sense to all cells and to some persistent cell-composed entities without brains or even nervous systems, where it is manifested as "basal cognition" (Lyon et al., 2021). Since, throughout this paper we have asserted that the cellular level is the fundamental locus of agency, there might be a sense in which intentionality also has an incipient form in free-living cells (see also Baluška et al., 2024).

The mathematical and computational approaches we have discussed are compatible with the two alternative possibilities of agency as a genuine, though elusive, phenomenon of living systems, or an apparent one, a function of our incomplete knowledge of what motivates organismal decisions. In either case, however, we are committed to the idea that the living systems that exhibit this property are subjects as much as they are objects of multiple levels of causation (Lewontin, 1983). We have therefore pointed to the need for novel dynamical models for organismal life trajectories that can be individual, idiosyncratic, and possibly elective.

We conclude the following:

(1) Organisms perform nonprogrammed, nonautomatic activities by which they relate to the external world, thereby showing that they have agency. Agency is characteristic of complex, multicellular entities, but increasingly recognized to also pertain to single cells. These include extant unicellular forms, but also social microorganisms that cycle between single- and

multicellular stages, the putative unicellular ancestors of animals, and transient unicellular components in cancers.

(2) The agency of multicellular entities derives from their cellular ancestors having been agential and their constituent cells being potentially so. The agency of cells is not a typical life-sustaining function – it has exploratory and creative aspects which are partly independent of survivability and, possibly, of selection in the history of their lineages. While cell agency appears entirely dependent on the integral organization (autopoiesis, closure of constraints) that underlies their autonomy, multicellular agency seems less strictly constrained and thus potentially open-ended and free.

(3) Not every manifestation of directionality or apparent purposiveness in multicellular entities is an indicator of agency. Cell masses can assume reproducible shapes and forms due to physical inevitability or inherency. A stereotypic response to an external stimulus, or stereotypic development, can be the result of natural selection. This makes evidence of agency difficult to disentangle from non-agential determinants in complex organisms and the embryos from which they develop. Social bacterial and amoebae, though phylogenetically distant from animals, and cancers, though pathogenic, may be better suited for discerning the role of agency in the transitions between single cells and many (and vice-versa).

(4) Clusters or aggregates of cells may or may not exhibit novel forms of agency. If they do, they can do it in several ways. They can express unicellular capabilities (ciliary motion, contraction, excitability), but in concert (as in "biobots"); they can enter into life-cycle-dependent transient associations with different stages having different exploratory relationships to their microenvironments (as in social microorganisms and tumors); or they can exhibit different individual-associated enablements and affordances during their lifetimes (as in animals). The emergence of entities with new forms of agency is accompanied by partial or complete suppression of the agency of constituent cells.

(5) Experimental analysis of unicellular-to-multicellular transformations and their reversals shows that multicellular forms can utilize structural and functional novelties, some transiently produced, to meet challenges not encountered in the evolutionary history of their species. Therefore, novelties which enable agential behavior need not have resulted from conventional selection for increasing fitness. New *morphological* enablements can be novel structural motifs readily accessed in the morphospace of the active, excitable biological matter of an earlier-

evolving developing system. In animals, novel *functional* enablements emerge with new cell types that arise from the subfunctionalization, partitioning, and amplification of conserved cellular functionalities that is characteristic of metazoans.

(6) Manifestations of agential behavior can be unprecedented or unpredictable, and the deviations from predictability might not be merely stochastic. A dispositional causal modality, rather than a strictly deterministic one, seems to be the appropriate philosophical framing of such phenomena. Correspondingly, the mathematical models developed for complex physical and chemical systems – for which unique outcomes (subject to stochasticity) follow from specification of initial conditions – might be more effectively replaced by models employing inexact differential forms, nonholonomic constraints, differential inclusions, and other formal features that provide natural openings for multilevel and multifactor determination, and emergent behaviors.

GLOSSARY

Adaptation The process by which Darwinian natural selection causes evolution by promoting retention or loss of variant organisms in a population. Also, resulting from this process, a trait that has been (generally gradually) brought about natural selection to meet an external challenge.

Affordance A feature of an organism's environment or ecological setting relevant to its specific capacities to perceive or act.

Agency Organism-initiated behavior that is species-characteristic but potentially unprogrammed and individually idiosyncratic.

Autonomy The property of a living system that enables it to produce and organize the components that compose it to establish its own goals and norms, and to promote the conditions of its existence through its interactions with the environment. Most theories of organismal autonomy consider autonomy a necessary condition of, but not identical to, agency.

Autopoiesis The characteristic, attributed to cells and more complex living systems, of being able to maintain their identity by producing and reproducing their own components and boundaries with the environments with which they interact.

Biomolecular condensates Micron-scale formations in the nuclei and cytoplasm of eukaryotic cells that lack surrounding membranes but concentrate biomolecules including

proteins and nucleic acids. Their physical properties, partly amorphous, partly gel-like, are poorly understood, but, among other processes, they mediate enhanced expression of functionally related genes during cell differentiation in animal embryos.

Closure of constraints A formal set of dynamical conditions claimed to be essential to autopoietic systems in which the work performed by each of an open system's energy-consuming processes creates constraints that channel their activities in a mutually dependent and self-sustaining fashion.

Darwinian unit of selection Any entity, e.g., a cell, a multicellular aggregate, an organism, that can reproduce and exhibit heritable variation in its capacity to meet external challenges.

Detachment Use of a pre-existing structure or property for something new. If feathers initially provided insulation and were much later used for flight, the second use reflects a detachment from the initial use.

Determinism The philosophical view that events are completely determined by previously existing causes. When applied to agency, it means that behaviors are not freely chosen or truly creative but are fully specified by a combination of prior factors, internal and external.

Dispositional causation Based on *disposition*, a tendency that may or may not be expressed in any given instance (related to *inherency*), an alternative concept to strict determinism, asserting that causation happens by a confluence of interacting entities and processes, with every event realizing a subset of the propensities, powers, or inherencies of the contributing factors.

Dynamical systems Systems with states that evolve according to specified relations among variables into subsequent states over time. They can be fully deterministic (described, for example, by networks of differential equations or logical functions) or have indeterminate outcomes, due to stochastic or chaotic effects or incomplete specification.

Emergence The phenomenon by which an entity or form of matter with new properties or regularities arises from the interaction of collections of material components (of one, or multiple kinds) with different properties. Examples include the formation of the elemental atoms from more basic particles, liquid water from H_2O molecules, and the evolution of developmentally capable animal tissues from unicellular (holozoan) progenitors.

Enablements Morphological or functional traits (e.g., appendages, organs) of an organism that help mediate its interactions with its environment. They contrast with *adaptations* in not

being assumed to have arisen in gradual steps to meet external challenges, and therefore not necessarily being outcomes of natural selection acting on individuals with different degrees of reproductive fitness. Some morphological novelties are optional enablements, e.g., the head crest of pigeons, the dorsal spines of sticklebacks.

Function A property of an organism that serves its survival, repair, reproduction, sensation or behavior. Examples include digestion, locomotion, excretion, vision. In unicellular organisms, function is represented in obligatory *functionalities* of subcellular component and organelles. In multicellular entities functions can be embodied in organs containing *differentiated* cells and can be optional enablements (speech mimicry in parrots, perfect pitch in humans).

Holozoans A clade of unicellular and transiently multicellular eukaryotic organisms with some extant representatives (e.g., choanoflagellates, ichthyosporeans) that are the closest relatives of the animals (the *metazoans*) and having presumptive direct common ancestors with the latter.

Inherency The characteristic dispositional properties of specific forms of matter (e.g., elasticity of solids, viscosity of liquids, propensity of water to generate waves and vortices, of animal tissues to form layers, cavities, segments).

Morphogenesis For multicellular organisms, the formation of structural parts (e.g., segments and appendages in animals, leaves and branches in plants, stalk and spore case in social amoebae) and their spatial relationships.

Morphospace The structural possibilities inherent to a kind of matter. The atoms of the chemical elements occupy the morphospace defined by protons, neutrons and electrons under certain physical conditions, and tissue layers, segments, and appendages are elements of the morphospace of metazoan cell aggregates.

Neoplasia Abnormal or uncontrolled growth of cells or tissues in the body, typically associated with cancer.

Nonholonomic system A physical system for which parameter changes that take it along a trajectory in its state space can end at a different state from the initial one when the parameter values return to the original ones. It can be represented by a set of expressions (such as inexact differential forms) for which the functions of the state variables are non-integrable. Systems having *nonholonomic constraints* have outcomes incompletely specified by the system's dynamics.

Novelty A new property, structure, or trait of an organism that permits it to perform a new function, invent new affordances, or construct and occupy a new ecological niche.

Scaffold In biology, a set of components or processes that serve as a framework for initiation or support, or a template, for the evolution or development of an organism or its parts. The scaffold can be structural, like those used in architecture, or processual, as with the combinations of oscillations and gradients of gene expression that scaffold the segments (somites) of the vertebrate embryo. The scaffold can disappear later in evolution, or as development proceeds.

Self-organization In physical processes, a property of some systems that are open to mass and energy fluxes in which persisting nonuniform structures (e.g., spots or stripes of chemical concentration) emerge out of a spatially homogeneous state. In biological processes, the emergence of complex spatial structures from a relatively unorganized mass of cells, e.g., during embryogenesis or carcinogenesis is also called self-organization. Biological structures can self-organize by physical processes when they first appear during evolution but, particularly as the system is transformed by genetic rewiring, biological self-organization becomes increasingly distant from the physical effects.

Spheroids Multicellular aggregates of cells suspended in culture medium that approximate the properties of tissues and tumors. They include embryoids, organoids, carcinoids, and "biobots." They exhibit some morphological and functional attributes of their source tissues as well as some novel properties. Where subject to appropriate assays (as in experiments with biobots) they manifest apparent agency. Some cancers (e.g., ovarian) employ a spheroid stage in their course of progression.

Teleonomy Biological processes, such as embryonic development or stereotypical behavioral routines that appear purposeful but are instead program-like products of evolution. Teleonomic processes can be contrasted with physics-based *teleomatic* ones, such as the formation of embryonic tissue layers by free energy-minimizing cell sorting, and *teleologic* ones in which authentically purposeful activities are presumably engaged by agential subjects.

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Protoc Cell Biol, Chapter 12:Unit 12.1.

FIGURE LEGENDS

Fig. 1 Life cycle of *Dictyostelium discoideum*, an aggregative eukaryotic microorganism. The circle on the left represents the *proliferation* that occurs in a nutrient-replete setting. The oval on the right shows the sequence of stages initiated under conditions of starvation: (clockwise, from top left), *exploration*, in which starved amoebae search for sources of nutrients, partly by random locomotion, but (by hypothesis) also via agential capabilities that could reflect learned experiences and idiosyncratic proclivities; *aggregation*, during which cyclic AMP-pulsing pacemaker cells, possibly randomly arising, but also potentially reflecting individual cell decisions, leads cells to respond with relayed signals and to move into liquid-like streams. The relay response and oriented movement have programmatic aspects that are products of evolution (i.e., teleonomic), and *directional streaming* is partly due to fluid dynamics (teleomatic), all of which may serve to limit cellular agency*; late aggregation and migrating slug*, in which multicellular entities appear to exhibit forms of agency, including directional movements, that differ from those of single cells, although individual cells in these masses exhibit divergent fates due to a combination of evolved tropic (chemotactic, haptotactic) responses and possibly agential behaviors; *stalk and fruiting body formation* result from oriented cell movements along with physical tranfomation (solidification) of the extracellular matrix. *Sporulation* is a partly programmed response in which the decision to enter this state may have both stochastic and agential elements. Amoebae emerge from spores after physically induced dispersal. (See main text and Arias Del Angel et al., 2020, from which the figure is adapted, for additional details and references.)

Fig. 2 Schematic depiction of the progressive stages of ovarian cancer metastasis within the peritoneal cavity. The process starts with the shedding of transformed epithelia (light green) from the ovarian capsule (A) into the ascitic fluid through a decrease in intercellular adhesion (reflecting teleonomic mechanisms) and as a result of shear stress due to peritoneal fluid movements (reflecting teleomatic principles). Within the fluid, epithelia exist in a unicellular state (B) or as multicellular sheets (C) (medium green). The latter has been proposed to form through aggregative tendencies of single cancer cells but also through detachment of de novo clusters of cancer cells. Such dysmorphic clusters rearrange themselves into organ-like organized

entities known as spheroids (D) with agentive behaviors like morphogenetic closure (an attenuation of the ability to allow cellular entry or exit, or the tendency (or decision) to coalesce with other spheroids), and emergent movements (temporal oscillations in size which aids survival in confined fluid compartments). These disseminated states ultimately colonize the peritoneal boundaries (E) through clearance of the defensive layers of mesothelia (light orange flattened cells) and form metastatic deposits (dark green stellate shaped cells). The formation of distinct multicellular organizations by transformed cells suggests a concept of cancer as a stepwise progression of agential transitions. Each multicellular pattern represents a neoplastic, or a novel morphological outcome that emerges through the interactions between the cells in the context of affordances provided by their metastatic microenvironment. (See main text for references.)

Fig. 1

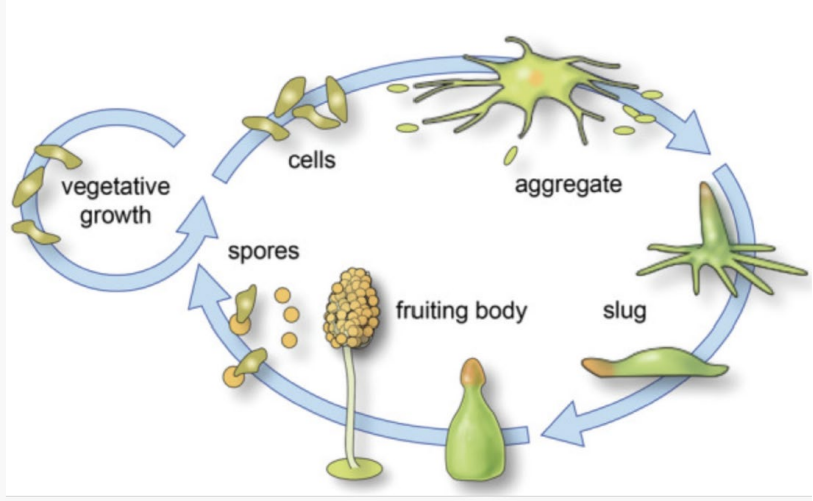
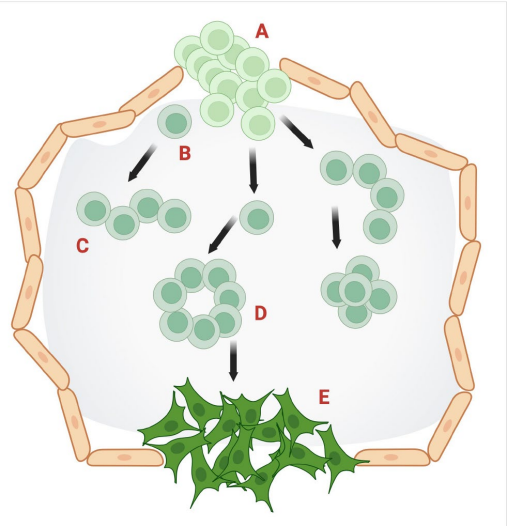

vegetative growth
cells
aggregate
spores
fruiting body
slug

Fig. 2


A
B
C
D
E