Agency in the Evolutionary Transition to Multicellularity

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Abstract

We discuss ideas of how *agency*, defined here as organism-initiated behavior (both species-characteristic and individually idiosyncratic), was aligned at the cellular and integrated in the multicellular levels during evolution. We consider agency in relation to the autonomy and purposiveness of cells and multicellular organisms. While the agency of cells in extant multicellular forms (and inferred in single-cell antecedents) is assumed in our analysis, we do not speculate on its origins. We attempt to discern the role of agency in the generation of form and function in social bacteria and amoebae, and we speculate on how these phenomena may relate to the emergence of phenotypically complex organisms. For the latter question, we explore the processes leading to morphological and functional enablements in metazoans and how these might change the character of organismal agency during evolution. We also consider how transitions between multicellular agency and unicellular agency (and back again) may characterize and drive the formation of cancers. We relate this problem to the philosophical discourse on dispositional causality and discuss experimental approaches to identifying genuine agency against a background of physically mediated directionality and evolved program-like behaviors of organisms. Lastly, we discuss the possible uses of mathematical representations of incompletely specified dynamical systems in the characterization of autonomy and agency.

Keywords: autonomy, determinism, dispositional causation, incompletely specified systems, inherency, physical scaffolding, social microorganisms

Introduction

Organisms have agency: at a minimum, they seek resources from their environment to promote their survival [1, 2]. 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 their environment changes. Such dispositions are transmitted to progeny in ways that are both type-specific and plastic. This review seeks to characterize how single-cell agency was integrated into multicellular developmental processes during the evolution of animals and other complex organisms, and how the agency of the developed organisms reflects these single-cell origins.

To set the boundaries of our review, we mention several important questions that pertain to agency at the single-cell level. In the context of evolutionary history, these are even more enigmatic than the evolution of multicellular development. Here, however, we propose to set them aside in favor of the difficult enough problem of transitions in the levels of agency.

We will therefore *not* focus on the following two questions:

(i) What is the full 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. Or perhaps to any and all forms of materiality [3]?

(ii) When did agency emerge in the history of the cosmos or the Earth? If (in answer to point (i)), cells are considered the ground state of agential matter, this question would be an empirical one of paleomicrobiology. But if some chemical systems have agency, was there a major transition in this respect that was prior to, or coincident with, the origin of life?

While questions (i) and (ii) are not irrelevant to our main concern of the unicellular-multicellular transition of agency we will not explore them in any depth. Instead, we will 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 [4, 5]). Specifically:

(iii) To what extent are behaviors exhibited by individual cells agential?

(iv) Does it make sense to distinguish between an organism's *being* an agent from its *manifesting* agency? That is, is an agent's agency always "on"?

(v) How was the ostensible agency aligned in cell collectivities 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?

(vi) What is the relationship between the integration of cell agency in multicellular evolution and its reversion, transformation, or repurposing in carcinogenesis?

(vii) 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?

(viii) 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 of an agential system?

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 intermediaries 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 "acts on its own behalf" [6], 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, is one pole of a range of views relating to what is experienced as free will in conscious organisms and degrees of self-motivated action in others [7]. The opposite pole is belief in willed action that is not completely constrained by antecedent physical determinants, with incipient counterparts of this down to the cellular level, that is, genuine agency [8]. A variety of intermediate positions are termed "compatibilism" [9]. 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.

While a motivating interest of this review is the evolution and development of animal multicellularity from its closest unicellular ("holozoan") ancestors to its reversals and reconstitutions in cancer, we mainly call on evidence and examples from other – noneukaryotic and nonholozoan – lineages, such as social bacteria and amoebae, in speculations on metazoan origination scenarios.

Finally, the questions discussed here involve a multiplicity of perspectives and disciplinary discourses, and unsurprisingly there are differences of opinion (even among the present authors, not each of whom is conversant with all the topics covered) that can run deep. We have tried to provide a balance of views (which are unlikely to be exhaustive) and note where important disagreements exist.

Agency at the cellular and organismal levels

While there have been multiple, often conflicting, proposed characterizations of agency (e.g., [8, 10-17]), we will assume for our analysis that a biological framing of agency consists of six independent properties:

(a) the ability of the entity ("self") to continuously demarcate itself from its environment ("non-self") and actively constitute and reconstitute its boundary [18].

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

(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 [19]) or inherencies [20] that may or may not manifest themselves. This means that a particular response to an environmental situation of an agential system may tend to go in a certain direction but need not do so [21, 22]. In other words, an agential system is one that has the capacity to respond to the same situation in more than one way. 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 "strong emergence" [21].[1] 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. The side-effect of sustained proximity is thus an evolutionary starting point of a novel multicellular agent. Analogous phenomena occur in the genesis of sociality in insects and even humans. The molecular basis of the binding of individuals will be different in each case, and is just the prerequisite for, not the defining character (which derives from the physical inherencies) of the new form of matter.

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 or agency 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 matter with altered inherencies (as described above) [23, 24].

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

[1] Strong emergence is familiar and relatively uncontroversial in cases like the atoms of the chemical elements forming from plasma as the universe cooled, or liquid water condensing from gaseous water molecules.

tissues in the body perform complementary functions via differentiated cells and organs. Cell assemblages that are integrated individuals can evolve in their form and function. Natural selection is based on different variants leaving different numbers of offspring in successive generations. The usual assumption (which may be violated; see [25]) 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 [26] or the colonization of novel ecological niches – out of competition with their relatives [27], and therefore independent of relative fitness.

Biological manifestations of agency

If cells ancestral to present-day animals exhibited agency, it is important to ask how it contributed to their lives differently from other biological properties. If agential acts are taken to involve choices or judgments of the moment, might it not be the case that the interests of living systems are best served by suppressing these in favor of fixed and stable response patterns? Posing the question this way suggests that while agential behaviors may be among the functional attributes of a living system [28], and even an indispensable one, not every function is a manifestation of agency. For example, navigation of a maze by the plasmodial slime mold Physarum [29] or pursuit of a mating partner by a sparrow could be characterized as agential activities. In contrast, the initiation of cell division when the amoeba reaches a certain mass, or the formation of segments in a bird embryo, are evolved functions that may not usefully be categorized as examples of agency, though a broader view might include all living functions under this rubric.

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. 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) [30], and therefore inescapable. Such processes have been termed “teleomatic” by Mayr [31].

Other goal-directed processes – those termed “teleonomic” [31, 32] – do not have this automatic character, but occur because they have evolved to operate in a directional fashion. 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 [33]. Teleonomic processes contribute to organismal survival by virtue of their highly reliable outcomes.

Many theorists believe that most if not all developmental and behavioral processes are deterministic in the teleonomic sense. The implication is that, except for a narrow range of variability due to inherent stochasticity, these processes are 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 true agency, as described above.

Here we provisionally pursue the view that genuine agency does, in fact, exist. 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 reproducible internal processes that are only loosely connected to species-characteristic behaviors. 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.

Moreover, 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. The idea of agency opens the door to a naturalized understanding of drives that reflect the propensity of living forms toward maintaining themselves through myriad means, not each of which need obviously favor maintenance. Curiosity may occasionally kill the cat (or more likely the kitten) but the proclivity for even risky or fallible explorations may well come with the nature of agency.

From unicellular to multicellular agency

According to the instrumentalist view considered, but set aside in the Introduction, a transition in agent-like behavior, e.g., from the unicellular to the multicellular state, might only involve a shift in the level of description. On one side of the shift there is a collection of entities that appear to behave like independent agents. On the other side, the set of those entities behaves like a single agent, a collective. The transition in apparent agency may or may not exhibit a steep dependence on the number of constituents, i.e., it may be smooth or abrupt. Also, the transition may be "noisy": the unit of agency may be poorly defined near its boundary with its environment. 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 new set of life-activities becoming significant to an observer.

If we attribute objective reality, and not just descriptive utility, to agency at the cellular level, is multicellular agency a straightforward consequence of being composed of cells or containing them, or is it something different? In population genetic terms, a multicellular entity would constitute a new unit of natural selection that is capable of contributing to reproductive fitness via heritable traits expressed at the collective level. Multilevel selection theory, e.g., [34-36], holds that the constitution of multicellular individuals is driven by natural selection based on the balance of how fitness plays out at multicellular vs. unicellular levels [37]. Thus, on one side of

the transition (as described above), there is a potential for conflict between individual fitness and collective fitness. But if agential capacities are potentially intensified by complexity and differentiation, would new forms of agency provide the multicellular state with an intrinsic advantage in this conflict?

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" [37].

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. A physical analogy is the viscosity of a liquid, which is not a property of its isolated subunits. 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 emergence of novel agential capacities can be seen, e.g., in the placozoan *Trichoplax adhaerens*. In this organism, motility and prey capture are enabled by ultrafast contractions of the dorsal epithelium, which is uniquely mechanically deformable among all animals because it lacks the restraining mesohyl matrix or basement membrane underlying the epithelia of sponges and other animals [38]. In addition, the cilia of the placozoan's ventral surface undergo a concerted behavior physically resembling the flocking of birds [39]. Neither of these activities (which enable new types of exploratory behavior) are found in the cells directly ancestral to the metazoans. Furthermore, both originate as self-organizing physical effects rather than as products of gradual evolution. (See the more detailed discussion in [40]). Novelties in other animal groups, such as nervous systems and brains, endow them with other emergent agential capacities, but they are more difficult to trace to physical effects.

The appropriation or alignment of cellular 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 automatic effect of new surface proteins or matrix molecules that were sticky or entrapping, or preexisting ones that acquired these properties with environmental changes. (Cadherins function as adhesive molecules only when ambient Ca^{2+} is sufficiently high, for example [41].) 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 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." Although the cellular subunits are living agents, their transition from individual to collective motion can partly be explained by the physics of phase transitions pertaining to nonliving systems [42]. The reverse physical transformation in tissues, analogous to a liquid-to-gas transition, is termed "epithelial-mesenchymal transformation" (EMT). Here a cohesive tissue becomes a collection of separate cells [43]. 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.

It is important to recognize that the novel forms of matter represented by multicellular aggregates, due to having subunits that are living cells, will be "excitable media" [44], "active matter" [45, 46]), or both.[2] They will thus have properties not readily predicted by physical laws formulated for conventional viscoelastic materials. By themselves, these dynamical supracellular attributes do not constitute forms of agency, 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 [47]. 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 [48], subpopulations of cells in the slug are differentially responsive to distinct external signaling molecules, and rearrange accordingly [49]. 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). 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

[2] 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.

state of differentiation. and possibly of agency, distinct from those of cells at other developmental stages.

One hallmark 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 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 relevant behaviors under experimental culture conditions, e.g., by navigating through mazes toward nutrients [50]. 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 is contrary to the suggestion that living organisms, when acting non-agentially, depart from this status [10].) 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 [51, 52], 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. These 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.

Agency in relation to purposiveness and autonomy

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 [53]. 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, but they do not produce the materials they comprise. They are examples of "self-organization" (a term first used by Kant to describe natural purposes) but of a purely physical (i.e., not biological) nature.

Physical self-organization (including the reaction-diffusion pattern-forming instabilities and other "dissipative structures"[3] described by Turing [54] and Prigogine and coworkers [55, 56]) contributes to the dynamics of living systems, and likely to their origins, but is different from biological self-organization [57, 58].

Empirically informed philosophical approaches to what Kant identified as intrinsic purposiveness (though Kant himself denied it was amenable to scientific analysis) were framed in terms of organization and nonequilibrium thermodynamics as the bases of living systems by the early to mid-20th century organicists, a collective of theoretical biologists that included Woodger, Needham, Waddington, and Bertalanffy (reviewed in [59-61]). Of particular significance is the notion of *autonomy*, discussed by Russell as early as 1930 [62]. 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" [63]. 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" ([64]; p. 78). The *organizational approach* of Moreno and 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 [6]. The "organization" of the organizational approach is "closure of constraints," in which mutually supportive work cycles construct one another's constraining conditions ([2], see also [65]).

The 19th and 20th centuries saw the recognition of the cell as the unit of life. As modern theories of autonomy, autopoiesis and the organizational approach were originally based on physically and chemically plausible, but abstract, organizational principles of this fundamental living unit. 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 [66] (though the definition of the latter is often controversial [67]). Transient colonies of social bacteria and amoebae (discussed above) are not individuals as conventionally understood, although their constituent cells at some life-cycle stages of fit the description. Further, the existence of eusocial organisms like bees and naked mole rats, and (with regard to language, for example) humans [68], 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 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.[4] As the examples above show, both agency and autonomy of cells in a

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

[4] 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 [69] C.M. Korsgaard, The constitution of agency : essays on practical reason

collective can 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 [70] – have extant forms with transient and constitutive multicellular morphotypes [71-73], 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" ([74], 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]" ([74], p. 333). Multicellular agency in this view would only pertain to eumetazoans, not the early diverging "basal" metazoans, sponges or placozoans, which lack both nervous systems and epithelia. It would certainly not apply to the biobots in the experiments described above [51, 52].

Without getting into too-detailed a comparison of the described perspective with conclusions presented by us here and previously [40], we suggest that the organizational approach features what seems to be an unduly stringent notion of functional integration to qualify multicellular entities as agents. The nine or so primitively specialized cell types of placozoans are not integrated into anything resembling organs (reviewed in [40]), and in locomoting and digesting bacterial prey these animals depend on cytological features no more elaborate than those found in ancestral cells [75].

Further, however, the autonomy and agency of even fully integral animals are not necessarily tied to genetic uniformity or species identity. Experimentally constructed embryo chimeras formed from the blastomeres of sheep and goats (estimated to have diverged 14-16 Mya), resulting in "geeps" [76], or from medaka and zebrafish (teleost lineages that separated on the order of 320 Mya [77]), 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, there is no requirement for them to participate in preexisting systems of closed constraints. As described in the next section, organismal agency appears to drive, rather than reflect evolution at the multicellular level.

Emergence of new enablements: reshaping of form and repurposing of function

In an agency-centered view of evolution (the organism as its subject, not as its passive object [1, 78]), traits are usefully considered *enablements* rather than *adaptations*. Adaptations are traits that evolve to meet the challenges of existing or changing environments better than those of

and moral psychology, Oxford University Press, Oxford ; New York, 2008.

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 [79]. Different shaped bird beaks suited to consuming different seeds are classic adaptations.

Enablements, by contrast, are features that could initially be novelties lacking specific functions, but later, when their bearers learn to use them for new ways of life, could become essential to the survival of the lineage and its individual members. Examples are body segments in multiple phyla, the antennae of insects, and the paired appendages of vertebrates. They can incidental outcomes ("spandrels") of developmental processes [80], or appear suddenly by modification via mutation ("hopeful monsters" *sensu* Goldschmidt [81]) or as side-effects of the readjustment [82] of such processes [82]. They will not be culled out immediately if they have a neutral impact on the organisms in which they appear [83], which will then be free to invent things to do with them [84-86].

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" ([87]; p. 119; see also [88]). 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*. The evolution of new morphological enablements occurs by effects that reshape and topologically reconfigure tissue masses during development [47, 89]. This can occur without the differentiation of new cell types, simply by the rearrangement of existing cells [90]. 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 [91, 92].

As noted above, the association of cells during development or evolution, by 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," gene products of the conserved "developmental toolkit" and the physical forces and effects they mobilize [93] elicit latent morphological propensities of the multicellular materials, leading to the formation of layers, interior cavities, segments, appendages, and external and internal skeletons [94]. While not themselves elements 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, and is a key symmetry-breaking event in the transition from unicellular to multicellular agency. It should therefore provide insights into the transformations of agency.

As noted above, placozoans survive and flourish with fewer than a dozen primitive cell types and no appendages or organs [75, 95]. 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 [51]. This means 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 [40, 96]). 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; [97]), and may therefore contribute to new forms of agency [40]. These novelties, initially optional, but ultimately lineage-defining and generatively entrenched [98], 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 [99], Walsh has asserted that agential beings evolve by using phenotypic novelties to invent new "affordances" [1].

More generally, the appropriation of essential cell functionalities to produce initially inessential but agency-enhancing capabilities in multicellular organisms occurs by a process of "detachment" [100] followed by subfunctionalization [101] via compartmentalization. Detachment entails establishing the independence of an aspect of an integrated whole, while subfunctionalization repurposes ("neofunctionalizes" [101]) that aspect. This combination of effects constitutes a different category of transformation from the self-organizational and other reshaping processes that appear to have driven the evolution of morphology.

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 [102]. 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 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 [103]. 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. The 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 [104].

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). This implies that agency is the capacity of a life-form (unicellular or multicellular) to be creative, and not something brought into existence by embellishments or additions (an epithelium, a nervous system) beyond what it takes to be alive. 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 and for identifying new environmental affordances [99].

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. With morphological innovation (based on the mesoscale physics of tissue masses), complex body plans emerged through anatomical compartmentalization (tissue layering, segmentation, appendage formation, and so on) and intensification of capabilities.

The rise of higher-order forms and functions can be usefully described as the emergence of a new "normative field" in which the more independent agency of individual cells has been forfeited in favor of a greater independence of the collective [105]. The capacity of individual cells to participate in such a trade-off may itself be an expression of cellular agency.

Cancer: transformations of multicellular agency

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 [106]. Thus, "sister" slugs, or two members of an animal species, may be more similar than cells within those multicellular forms. Correspondingly, at the lower level of agency there could be more homogeneity between sister cells than between the molecular and physiological regimes within cells. The propensity of cells, multicellular tissues/organs, organisms, etc., to realize a normativity, a higher-level ordering that supervenes over internal heterogeneity, may be a defining condition of natural agency [105].

In the opposite causal direction, it would follow that neoplasia or cancer begins when the ability of a tissue to normatively scaffold or to override the heterogeneity of constituent cells lapses, such that rather than continuing to achieve agential integration at the tissue level, a cell (or cells) begins to reestablish its individuating cell-agency, detaching it from the tissue-level regime [105].

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 [107]. These, in turn, acquire novel morphological enablements through physical self-organization [108, 109] 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.

The pathological reversals of cancer (by symmetrical inference) may provide insight into multicellular origins. 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, for instance, could differ from those of individual cells in part because some level of differentiation between cell types may be constitutive of what a larger form can do. So long as the latter can retain the functional unity of the sum of its cells, it can afford to engage in higher risk exploration that may pay off or may not. This is amenable to experimental investigation using transient multicellular systems like social bacteria or amoebae. In what sense, for example, does the slime mold slug explore the environment differently than the individual cell?

Experimental challenges in the characterization of agency and its transformations

Empirical and experimental approaches are crucial for the validation, enrichment, and expansion of the philosophical discussions around agency. 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 [23, 110].

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 [40]. 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 [50, 111] or multicellular aggregates [51, 52] 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 challenge is to select appropriate biological models and to delineate their advantages and limitations. For instance, slime molds and myxobacteria are good models to study the development of multicellularity by aggregation (e.g., [42, 112, 113]), while yeasts and cyanobacteria can be used to study the transition to multicellularity in clonal or "staying together" scenarios (e.g., [114, 115]).

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[5] 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 [42]). The volvocine algae provide paradigmatic examples of these features [117, 118]. In utilizing these systems, however, it is important to avoid interpretations that appeal to "living fossils" or "ladder [of progress] thinking" [119].

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. This implies relevant biases and presents technical, methodological, and analytical challenges that are being addressed by a variety of strategies [120].

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 processes that produce them (e.g., [109, 113, 121]).

A third challenge in studying the transition to multicellularity with an agency-informed perspective is of 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 [120, 122]. 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.

[5] The Reynolds number is defined as the ratio of the inertial forces to the viscous forces of a fluid [116] E.M. Purcell, Life at low Reynolds number, Am. J. Phys., 45 (1977) 3-11.

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 [123]), 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.

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 [123, 124]. 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 a fairly advanced – meaning relatively recently evolved – species [125-127], 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 bias just alluded to has 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 ([128]; 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 with regard to developmental details, considering robustness of the broad features of the life cycle [129], this possibility cannot be ignored.

Plasticity within species can mimic characteristic features of other species. Multicellular morphologies show back-and-forth phylogenetic transitions [130] 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" [131]. Because aggregative multicellularity has evolved independently at least five times in the six eukaryotic supergroups (or eight times depending on how one counts [132]), 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 coming up with experiments on *D. discoideum*, primarily and *D. giganteum* and one or two other CSMs, secondarily. Two questions require 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 [133].

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 [134], 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 ([112, 123, 124, 130]. 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 (the organism as an object of evolution, per Lewontin’s formulation) to organism-initiated factors (as evolution’s subject) [78]. 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.

Cells in the embryos of extant metazoans are typically scaffolded by mesoscale physical effects and do not behave as independent agents during development [135]. Cancer, however, 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 [136].

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 [137]. 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 [138]. 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 [109, 139].

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 [140]. Care must be taken, 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 [141]. 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 [142]. Attalla and coworkers showed that expression of a variant of the human epidermal growth factor 2 (HER2) receptor promoted a cold TIME and thus increased the spread of the tumor. The variant is not a somatic mutation, but an alternative splice form resulting from a changed balance of RNA splicing factors internal to the cell. Surprisingly, some of the factors induced by, or associated with, the expression of the variant were proteins whose abundance were controlled post-transcriptionally, i.e., by differential use, not expression of the cognate mRNAs [141]. While the causal chain leading to these cold TIME-inducing changes might, in principle, be identified, if a role for a tumor’s agency in advancing its own fate indeed exists, it might be found in such venues and phenomena.

Mathematical and computational modeling of putatively agential systems

Biological agency may elude strict determinism. 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 entirely, 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 [143]. 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 [144].

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 without uniform 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 [145]. Slime mold aggregation can also be modelled as the consequence of an instability that sets in beyond a critical spatial density [146]. This model is capable of reproducing several characteristic features of *D. discoideum* aggregation, including the formation of inwardly streaming cells [147], which raises the interesting possibility that agent-like behavior (and a discrete mode of description) may have been enabled during evolution after an initial transition from a dispersed single cell state to a multicellular collective (see previous section).

Our discussion earlier in this review, however, 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 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 [25] 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*" [148].

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 [149, 150].

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.[6] 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 $h(x_1, \ldots, x_n)$, the constraints are thereby holonomic and the inaccessible regions of state space increase, i.e., fewer variables are needed to describe its state.

Inexact differential representations can be useful in a practical sense in systems or subsystems in which there is insufficient information to derive a complete set of dynamical equations. 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,

[6] See [151] C. Hooker, On the import of constraints in complex dynamical systems, Foundations of Science, 18 (2013) 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 [152, 153]. 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 [21, 22] (see the discussion above).

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 [154], 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 [155], 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 containing elements of intrinsically 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 [156, 157] 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 [158-160], applications to the life cycle of cellular slime molds [161], multicellular bacteria [113], and tumorigenesis [162] demonstrate its relevance to phenomena where agency may play a more prominent role.

Discussion

Agency joins an increasing number of topics – 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, past 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 nuclei 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.

To these, however, we can add sources of uncertainty that go beyond randomness. The determinants of living systems extend beyond the above-mentioned "recognized principles of physics and chemistry." The materials represented by multicellular aggregates (variously "excitable media" [44] and "active matter" [45, 46], 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 intrinsic disorder to varying extents [163] (defying Anfinsen's famous principle of sequence-structure determinism) and constitute, along with noncoding 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 [164].

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 [2, 64] and quasi-chemical models for its realization [165, 166] 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" [167]. This includes those that derive from autopoiesis and the organizational approach, not discussed here, but also, to the extent that we refer to cell-level phenomena, the synthesis presented in this paper.

In contrast to the origin of 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 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 [20], 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, those discussed here – which draw on jumps between morphological inherencies or partitioning of preexisting functionalities – persist only in relation to the agency of the organisms that carry them. They are "Kauffman's screwdrivers" [87], 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 [168]. 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.

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 [21, 22]. Anjum and Mumford [21, 22] 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" ([21]; p. 80). They propose

that "science should be about uncovering the real causal powers of things as evidenced in their tendencies," [21], p. 138).

Dispositional notions relevant to agency are autonomy and *intentionality* ([21] p. 151). Autonomy, discussed above, is a fundamental property of cells (though it can be relinquished by them, and its physico-chemical basis and evolutionary origins are not understood), but not necessarily of all organisms, as seen in the examples of social bacteria and cellular slime molds, discussed above.

Intentionality, which is the property of being directed toward some object or situation and contains the implication of deliberate choice, is an ability of "minded creatures," according to Anjum and Mumford ([21], p. 152). While the attribution of mindedness is beyond the scope of this paper, there is evidence that it pertains in some sense to all cells and some persistent cell-composed entities without brains or even nervous systems, where it is manifested as "basal cognition" [111]. 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.

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 [78]. We have therefore pointed to the need for novel dynamical models for organismal life trajectories that can be individual, idiosyncratic, and possibly elective.

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 (both species-characteristic and individually idiosyncratic).

Autonomy The property of a living system that enables it to produce and organize the components that compose it so as 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 it to encompass agency.

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, the mediate enhanced expression of functionally related genes

during cell differentiation in animal embryos.

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*), a concept alternative to strict determinism, proposing 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 system Systems with well-defined states that evolve 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.

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 productive interactions with its environment. They contrast with *adaptations* in not being assumed to have arisen (generally gradually) to meet external challenges, and therefore not necessarily being outcomes of natural selection. The appearance of novel enablements can create novel affordances, for example.

Function A property of an organism that serves it 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).

Morphology For multicellular organisms, the 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. The generation of morphology is called *morphogenesis*. Some morphological components are optional enablements (the head crest of pigeons, the dorsal spines of sticklebacks).

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

Nonholonomic system A physical system whose state depends on the path taken in order to achieve it. It can be represented by a set of expression (such as inexact differential forms) for which the functions of the state variables are non-integrable. This implies that changes in the system's parameters that take the system along a trajectory in its state space can end at a state different from the initial one even if the parameter values return to the original ones. Systems having *nonholonomic constraints* have incompletely specified outcomes.

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 an architectural one, or processual, as with 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 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. Biological self-organization can be scaffolded by physical self-organization during evolution or development but is mechanistically distinct from it.

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 engaged by agential subjects.

Acknowledgments

We thank Lenny Moss for conceptual contributions to, and critical comments on, this paper, and Jim Siegel for expert editorial suggestions. The authors gratefully acknowledge the financial

support of the John Templeton Foundation, United States (#62220). The opinions expressed in this paper are those of the authors and not those of the John Templeton Foundation.

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