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Interplay of mesoscale physics and agent-like behaviors in the parallel evolution of aggregative multicellularity

Juan A. Arias Del Angel,^{1,2,3,4†} Vidyanand Nanjundiah,⁵ Mariana Benítez,^{1,2} and Stuart A. Newman³

¹Laboratorio Nacional de Ciencias de la Sostenibilidad, Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad de México, México

²Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Ciudad de México, México

³Department of Cell Biology and Anatomy, New York Medical College, Valhalla, NY 10595, USA

⁴Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México, México

⁵Centre for Human Genetics, Electronic City (Phase I), Bengaluru 560100, India

†d. 2019

Contact: mбенitez@ieciologia.unam.mx (MB); vidyan@alumni.iisc.ac.in (VN); newman@nymc.edu (SAN)

22 **ABSTRACT**

23 Myxobacteria and dictyostelids are prokaryotic and eukaryotic multicellular lineages,
24 respectively, that after nutrient depletion aggregate and develop into structures called fruiting
25 bodies. The developmental processes and the resulting morphological outcomes resemble one
26 another to a remarkable extent despite their independent origins, the evolutionary distance
27 between them and the lack of traceable levels of homology in the molecular mechanisms of the
28 groups. We hypothesize that the morphological parallelism between the two lineages arises as
29 the consequence of the interplay, within multicellular aggregates, between *generic processes*,
30 physical and physicochemical processes operating similarly in living and non-living matter at the
31 mesoscale ($\sim 10^{-3}$ - 10^{-1} m) and *agent-like behaviors*, unique to living systems, characteristic of
32 the constituent cells. To this effect, we analyze the relative contribution of the generic and
33 agent-like determinants in the main phenomena of myxobacteria and dictyostelid development,
34 and their roles in the emergence of their shared traits. We show that as a consequence of
35 aggregation collective cell-cell contacts mediate the emergence of liquid-like properties, making
36 nascent multicellular masses subject to new sets of patterning and morphogenetic processes. In
37 both lineages, this leads to behaviors such as streaming, rippling, and rounding up, similar to
38 effects observed in non-living fluids. Later the aggregates solidify, leading them to exhibit
39 additional generic properties and motifs. We consider evidence that the morphological
40 phenotypes of the multicellular masses deviate from the predictions of generic physics due to
41 the contribution of agent-like behaviors. These include directed migration, quiescence, and
42 oscillatory signal transduction of the cells mediated by responses to external cues acting
43 through species-specific regulatory and signaling mechanisms reflecting the evolutionary
44 histories of the respective organisms. We suggest that the similar developmental trajectories of
45 Myxobacteria and Dictyostelia are more plausibly due to shared generic physical processes in
46 coordination with analogous agent-type behaviors than to convergent evolution under parallel
47 selection regimes. Finally, we discuss the broader implications of the existence and synergy of
48 these two categories of developmental factors for evolutionary theory.

49
50 **Key words:** myxobacteria; dictyostelids; liquid tissues; deformable solids; excitable
51 media

52 INTRODUCTION

53 The emergence of multicellular organisms exhibiting cell differentiation, spatial patterning and
54 morphogenesis has been recognized as one of the major transitions in evolution (Maynard
55 Smith and Szathmáry, 1995). Depending on the criteria applied (cell–cell attachment, cell
56 communication, division of cell labor, among others) multicellularity evolved on anywhere
57 between 10 and 25 independent occasions (Niklas and Newman, 2013; Niklas and Newman,
58 2019). The appearance of multicellular organisms enabled an extraordinary increase in the
59 complexity of living systems and the study of the developmental mechanisms and selective
60 forces leading to its emergence, maintenance, and variation is an active research area (e.g.,
61 Niklas and Newman (2016). In broad terms, multicellular organisms can be classified either as
62 aggregative (“coming together”) or zygotic (“staying together”), according to the mechanism by
63 which multicellularity arises (Bonner, 1993; Tarnita et al., 2013). In the former, multicellular
64 organisms develop through the gathering of several individual cells potentially belonging to
65 different genetic lineages; in the latter, all the cells in the organism are the offspring of a single
66 cell and remain attached to each other after cell division (Bonner, 1998; Grosberg and
67 Strathmann, 2007). Across eukaryote lineages, aggregative multicellularity involves amoeboid
68 cells and leads to the formation of a fruiting body or “sorocarp” (Brown and Silberman, 2013).
69 There appear to be ecological determinants (e.g., resource availability, land vs. water
70 environment) of whether organisms are clonal or aggregative (Bonner, 1998; Fisher et al., 2019;
71 Hamant et al., 2019). Furthermore, clonal lineages do not always exhibit complex development
72 with different cell types and arrangements, and aggregative ones often do (Newman, 2014b;
73 Newman, 2019c; Niklas and Newman, 2019).

74 Dictyostelia and Myxobacteria are eukaryotic and prokaryotic multicellular lineages,
75 respectively (Romeralo et al., 2013a; Yang and Higgs, 2014). In these lineages, the life cycle
76 comprises a vegetative and a developmental stage. In the vegetative stage, Dictyostelia behave
77 as solitary cells acting independently of each other, and with the possible exception of
78 intercellular repulsion during feeding (Keating and Bonner, 1977), only engage in cell-cell
79 interactions during development. In contrast, Myxobacteria, often referred to as social bacteria,
80 are believed to organize into cell consortiums through their entire life cycles, although single-
81 cell-specific behaviors are observed in the laboratory (Thutupalli et al. (2015) and unpublished
82 observations). Both lineages are commonly found in soils where they feed upon (other) bacterial
83 species. Once nutrients have been depleted, they transit into a developmental stage
84 characterized by a substratum-dependent cellular aggregation that culminates in the formation

85 of multicellular structures called fruiting bodies, containing up to 10^5 - 10^6 cells, where cell
86 differentiation takes place (Whitworth, 2008).

87 The basis of cell differentiation in *D. discoideum* has been explained in two ways. There are
88 pre-aggregation tendencies among amoebae, stochastic in origin, biased by the environment
89 they experienced during the phases of growth and division, or, cell differentiation is a post-
90 aggregation phenomenon based on intercellular interactions and diffusible morphogens
91 (reviewed in (Nanjundiah and Saran 1992). There is experimental evidence for each of the two
92 viewpoints (Kawli and Kaushik, 2001), and it is also clear that subsequent interactions can
93 override cell-autonomous tendencies (Raper, 1940).

94 In Myxobacteria development, cells commit to at least three different cell types, peripheral
95 rods, spores and autolysis. In Dictyostelia, there are principally only two terminal cell types, stalk
96 and spore cells, with several transitory cell types (different pre-stalk and pre-spore subtypes)
97 observed over the normal course of development. Phylogenetic analyses suggest that the
98 capacity for cellular differentiation predated the emergence of multicellular development in both
99 lineages (Arias Del Angel et al., 2017; Schaap et al., 2006). Theoretical studies show that
100 cellular differentiation can spontaneously arise by the coupling of multistable cellular systems
101 (Furusawa and Kaneko, 2002; Mora Van Cauwelaert et al., 2015).

102 The morphology of fruiting bodies in both lineages displays a similar extent of diversity
103 ranging from simple mound-like to highly branched tree-like structures. Morphology is a species-
104 dependent trait, though there are examples in the dictyostelids of the fruiting body of one
105 species mimicking the morphology of another (Bonner, 2009). For neither Myxobacteria nor
106 Dictyostelia are fruiting bodies morphologies a monophyletic trait (Arias Del Angel et al., 2017;
107 Schaap et al., 2006), and thus different forms are likely to have evolved multiple times within
108 each lineage.

109 The issue of convergence becomes even more remarkable when it is recognized that
110 sorocarpic amoebae like those of Dictyostelia occur in five of the seven supergroups into which
111 eukaryotes are divided. (Archaeplastids, the group containing red algae, green algae, and
112 plants, appear to be the sole exception. In another supergroup, the Alveolates, aggregative
113 multicellularity and fruiting body formation occurs, but in ciliates, not amoebae (Bonner, 2009;
114 Brown and Silberman, 2013).

115 Perhaps more surprising is the resemblance of developmental processes and resulting
116 morphologies between eukaryotic sorocarpic amoebae such as Dictyostelia and the prokaryotic
117 Myxobacteria, despite their independent origins, the evolutionary distance between them, and
118 the lack of traceable homology in the molecular mechanisms in each group (Fig. 1). Bonner

119 (1982) suggested that the parallelisms between Myxobacteria and Dictyostelids appear as a
120 consequence of either similar selective pressures or shared developmental constraints. But
121 these determinants are not mutually exclusive and discrimination between them is not trivial
122 (Olson, 2012). Kaiser (1986) proposed that a joint investigation of Myxobacteria and Dictyostelia
123 could potentially lead to the identification of generalities underlying the multicellular phenotypes
124 across both lineages.

125 Since Kaiser's proposal, a combination of experimental and modeling approaches has been
126 employed to investigate the development in these two lineages (Romeralo et al., 2013b; Yang
127 and Higgs, 2014). Such studies advanced after physico-chemical processes came to be
128 considered as key factors determining the developmental outcomes (Bretschneider et al., 2016;
129 Fujimori et al., 2019; Thutupalli et al., 2015; Umeda and Inouye, 2002). Specifically, there is a
130 recognition that the shaping of multicellular masses cannot be explained independently of their
131 material properties, and that developing organisms are thus subject to physical forces and
132 effects relevant to their composition and scale (Benítez et al., 2018; Newman, 2014a; Newman
133 and Bhat, 2009; Rivera-Yoshida et al., 2018). When applied, for example, to embryonic animal
134 tissues, which behave similarly, in certain respects, to non-living liquids and liquid crystals,
135 physical models predict the formation of immiscible layers, interior spaces, and, when the
136 subunits are anisotropic, the capacity to undergo elongation (Newman and Bhat, 2009). In
137 contrast, plant tissues, characterized by rigid cell walls, behave like deformable, mechanically
138 and chemically active solids which (unlike liquid-state materials) can bud or branch (Benítez et
139 al., 2018).

140 Properties shared by cellular masses with (as the case may be) nonliving liquids, solids, or
141 semisolid materials have been termed "generic" (Newman and Comper, 1990), and we adopt
142 that term here. The physical forces, effects and processes inherent to such materials enable
143 and constrain developmental outcomes in multicellular masses, leading to the conclusion that
144 homoplasy (the same form, independently evolved) is expected to be common, and some
145 morphological motifs should be recurrent and predictable (Benítez et al., 2018; Newman,
146 2014a). Physical determinants, in this view, are complementary to the regulatory dynamics
147 within cells. Indeed, physical and physicochemical processes are mobilized on the multicellular
148 scale by genes, their products and other molecules, and are thus subject to regulation
149 throughout evolution (Benítez et al., 2018).

150 In contrast to the molecular subunits of non-living materials, the individual cells constituting
151 a multicellular cluster are able to sense and respond to local cues through signaling and
152 regulatory pathways. Because of their intracellular chemical dynamics and capacity to generate

153 mechanical forces, cells can be understood as agents that actively modify their behavior in
154 response to their environment, and even modify their environment in ways that can further affect
155 the cell-environment interaction. These processes taking place at the cell level, including
156 chemotaxis, which as discussed in Section 4, can continue even when the cells are already
157 aggregated, can translate into collective behaviors that act in parallel and coordination with, and
158 even oppose, the generic physical processes that shape a tissue mass. These “agent-like”
159 behaviors modify the outcomes that would be expected if only generic physical processes were
160 operative.

161 Here, we hypothesize that the morphological outcomes, and thus the parallelism between
162 the myxobacterial and dictyostelid lineages, originated as a consequence of the interplay
163 between generic processes acting upon the multicellular materials and agent-like behaviors
164 characteristic of the constituent cells. To this end, we describe the major generic and agent-like
165 properties exhibited during the development of these lineages and attempt to analyze their
166 contributions to the emergence of the groups’ shared traits. We suggest that as a consequence
167 of aggregation, the nascent multicellular mass becomes subject to new sets of patterning and
168 morphogenetic processes resulting from the fact that cell-cell contacts or immersion in a matrix
169 mediate the emergence of a fluid-like properties. In both lineages, this leads to developmental
170 processes, e.g., streaming, rippling, that are similar to behaviors observed in non-living fluids.
171 We explore the idea that deviations of the dynamics and morphological outcomes of the
172 multicellular mass from the generic predictions are due to the contribution of agent-like
173 behaviors of individual cells, e.g., gradient sensing, directed migration, quiescence.

174 Generic effects are *common causes* in the different lineages. This is because whatever
175 molecules underlie the realization of properties such as cell-cell adhesion, spatial heterogeneity
176 via diffusion gradients, and so in in different lineages, the morphological outcomes are similar by
177 virtue of being produced by similar physical generative processes. Agent-behaviors, in contrast,
178 are peculiar to disparate lineages (cell locomotion, for example, has very different physical and
179 genetic bases in prokaryotes and eukaryotes, as does entry into the quiescent state), reflecting
180 the evolutionary histories of the respective organisms. However, these behaviors can be
181 analogous to one another, thus contributing to convergent morphological outcomes. Further,
182 analogous intracellular dynamical behaviors such as biochemical oscillation can be organized
183 by generic effects such as synchronization, leading to additional shared generic modes of
184 organization. We conclude that the similar developmental programs of Myxobacteria and
185 Dictyostelids are plausibly due to shared generic physical processes in coordination with
186 analogous agent-like behaviors.

187

188 **GENERIC MATERIAL PROPERTIES OF MYXOBACTERIAL AND DICTYOSTELID**
189 **MULTICELLULAR MASSES**

190 Based on the observation that animal life is characterized by a restricted set of basic forms and
191 patterns, Newman and co-workers advanced the conceptual framework of “dynamical patterning
192 modules” (DPMs). DPMs are defined as sets of gene products and other molecules in
193 conjunction with the physical and physicochemical morphogenetic and patterning processes
194 they mobilize in the context of multicellularity (Newman and Bhat, 2008; Newman and Bhat,
195 2009). These include phenomena such as adhesion and differential adhesion, and reaction-
196 diffusion effects. This framework emphasizes that the material nature of a developing organism
197 makes it subject to generic physical processes (i.e., those common to living and nonliving
198 viscoelastic and excitable systems) and that they readily exhibit morphological motifs – layers,
199 segments, protrusions – Inherent to the respective materials. The term “module” is employed to
200 highlight the semi-autonomous action of DPMs in determining specific spatial patterns and
201 structures. But the DPMs also interact during development and can thus be conceptualized as a
202 complex “pattern language” for generating organismal form (Hernández-Hernández et al., 2012;
203 Newman and Bhat, 2009). This approach is distinguished from a purely “tissue physics”
204 framework since it also recognizes the relevance of the cells as repositories of genetic
205 information, making such systems subject to evolutionary processes not applicable to non-living
206 matter.

207 Even when the similarity in the mesoscopic (i.e., physics of the middle scale) properties of
208 living and certain kinds of non-living matter is recognized, it should not be taken to imply that
209 they are constituted in the same way. The liquid or solid nature of living tissues does not arise
210 from the same subunit-subunit interactions that endow non-living materials with these
211 properties. This is particularly the case with the liquid-like state of animal tissues. Instead of the
212 thermal vibration- driven Brownian motion that causes the molecular subunits of non-living
213 liquids to move randomly, the cells in animal tissues move actively by ATP-dependent
214 cytoskeleton-generated forces, which in the absence of external signals is also random. Despite
215 continually changing their neighbors, subunits of nonliving liquids cohere due to the weakly
216 attractive electronic interactions that hold them together. The cells of developing animal tissues
217 also remain cohesive despite their translocation, but for a different reason: the homophilic
218 attachment proteins (classical cadherins) that mediate their transient attachment extend through
219 the cells’ membranes to form stable connections between adhesive and motile functions
220 (Newman, 2019b). In plant and fungal tissues, instead of the charge-based or covalent bonds of

221 the atomic or molecular subunits of non-biological solids, the cells are cemented together by
222 pectins and glycoproteins which are subject to unique forms of reversible remodeling (Benítez et
223 al., 2018; Hernández-Hernández et al., 2012). Because these generic properties are dependent
224 on evolved biological, rather than purely physical effects, the various viscoelastic and
225 deformable solid materials that constitute living tissues have been termed “biogeneric” matter
226 (Newman, 2016).

227 In the following, we describe some of the generic and biogeneric properties and processes
228 of Myxobacteria and Dictyostelia multicellular masses and compare these properties to those
229 implicated in animal development. Then, we describe the molecular components that establish
230 and mobilize these properties in both Myxobacteria and Dictyostelia. Next, we highlight some
231 developmental phenomena in these organisms and evaluate the extent to which these can be
232 explained by generic physical behaviors, and what is left unaccounted for. Finally, we describe
233 the agent-like behaviors of the subunits (bacteria and amoeba) of the two systems, discuss their
234 similarities and differences, and discuss how analogous agent behaviors coordinate with and
235 complement the described generic properties, and potentially account for the common
236 developmental modes of Myxobacteria and Dictyostelia.

237

238 **Adhesion- and matrix-based cell-cell association**

239 Cell adhesion is the defining characteristic of multicellular organisms and the nature and
240 strength of cell bonding is a major determinant of tissue properties (Forgacs and Newman,
241 2005; Mora Van Cauwelaert et al., 2015; Niklas and Newman, 2019). In animals, cell-cell
242 adhesion is mediated by membrane proteins such as cadherins that permit cells to be
243 independently mobile and capable of moving relative to another while remaining cohesive. As
244 noted above, the animal tissues from which embryos and organs develop behave formally like
245 liquids (Newman, 2016).

246 In *D. discoideum*, cell-cell adhesion at early stages of development involves the action of
247 several proteins including the immunoglobulin-like DdCAD-1 and the glycoproteins gp80 and
248 gp150 whose expression and activities are tightly regulated during the different stages of
249 development (Coates and Harwood, 2001). Later in development, when cells have entered into
250 streams and cell density has increased, the cells are also embedded in cellulose-based
251 matrices that provide the basis for adhesion in cellular conglomerates (Huber and O'Day, 2017).
252 In the case of *M. xanthus*, persistent cohesion is correlated with the secretion of thick fibrils,
253 composed of carbohydrates and proteins, that coat the cell surface and constitute an
254 extracellular matrix that interconnects the cells (Arnold and Shimkets, 1988; Behmlander and

255 Dworkin, 1994a; Behmlander and Dworkin, 1994b). Chemical or genetic disruption of fibrils
256 causes defects in agglutination and failures in social and developmental behaviors. In a similar
257 fashion to the animals, cell-cell adhesion in Myxobacteria and Dictyostelia depend, to different
258 degrees, on the presence of divalent cations (Lin et al., 2006; Shimkets, 1986).

259 Both Myxobacteria and Dictyostelia also have strong associations with external substrata
260 during their pre-culmination stages of development. The closest analogy in animal systems is
261 the interaction of cell layers in eumetazoans with internally generated planar basal laminae,
262 which are not generally present in the earliest diverging and morphologically simplest
263 metazoans, sponges and placozoans. In both Myxobacteria and Dictyostelia cells are more
264 loosely associated with one another as they interact with the substratum than are the cells in
265 planar animal epithelia. In the non-animal systems, cell substratum interactions depend on focal
266 adhesions that indirectly (in contrast to directly in animal tissues) mediate communication
267 between the substratum and the actin cytoskeleton, where they also provide the foundation for
268 cellular motility (Faure et al., 2016; Fukujin et al., 2016).

269 A key difference between the respective lineages is that dictyostelid cells only engage in
270 persistent cell-cell interactions shortly after starvation, whereas extensive cell-cell adhesion and
271 interactions take place among myxobacterial cells through their entire life cycle. While the
272 mechanisms involved in cell-cell and cell-substratum contact between Myxobacteria and
273 Dictyostelia are different, in both cases the bonds between adjacent cells are weak enough to
274 allow cells to rearrange relative to one another during aggregation and shortly after mounds are
275 formed. Therefore, aggregating cells in these lineages behave like non-living liquids, exhibiting
276 streaming and rippling behaviors characteristic of such materials. This contrasts with
277 monolayered animal tissues (epithelia) which, though also having liquid-like properties in the
278 plane, bind too strongly to their intra-organismal substrata, *basal laminae*, to manifest similar
279 fluid-like behaviors at the planar interface (Mittenthal and Mazo, 1983).

280 Unlike Dictyostelia, in Myxobacteria some type of cell-cell adhesion or matrix embedment is
281 present throughout the whole life cycle, causing cellular masses to exhibit liquid-like behaviors
282 in both vegetative and developmental stages (Thutupalli et al., 2015). During predation, cells
283 align and move concertedly into ripple-like travelling waves (Zhang et al., 2012). Once
284 development has started, *M. xanthus* aggregation is largely driven by entropy minimization
285 through reduction of the surface area on which the collective cell population contacts the
286 substratum (Bahar et al., 2014). This is a comparable behavior to that of liquid droplets, where
287 individual subunits or clusters move into larger droplets of larger volume but smaller contact
288 area with the surface. In Myxobacteria, phase separation has not been implicated in sorting of

289 cell types inside fruiting bodies. However, since spores are coated by material that increases
290 cell cohesiveness, differential adhesion likely contributes to the spontaneous sorting out of
291 spores from peripheral rod cells, reflecting their liquid-like properties.

292 It is important to distinguish the liquid-like properties of both Dictyostelia and Myxobacteria
293 cell streams and masses from that of embryonic animal tissues. In epithelioid animal tissues the
294 cells are directly attached to their neighbors by transmembrane cadherins which maintain strong
295 cohesivity while permitting rearrangement. This is consistent with persistent apicobasal
296 polarization that allows for the formation of lumens within cell masses, and planar cell
297 polarization that permits elongation and other reshaping of tissues by intercalation and
298 convergent extension, a liquid-crystalline like phase transformation. In Dictyostelia, the cells are
299 embedded in cellulose-based matrices that enable cell rearrangement and hence the liquid-like
300 behaviors described above (Huber and O'Day, 2017). However, the lack of direct engagement
301 in this attachment mode with the cytoskeleton makes cell polarization, even when it occurs,
302 transient and unproductive to lumen formation or stable intercalation (Manahan et al., 2004;
303 however, see Hayakawa et al. (2020)). Cells of Dictyostelia also have a more pronounced
304 chemotactic response to extracellular signals than most animal embryonic cells, which
305 contributes to their particular version of liquid-tissue properties (Tan and Chiam, 2014) (see
306 below).

307 The glycoprotein-based associations of Myxobacterial cells are also too transient, and their
308 polarity too rapidly reversible, to allow lumens to form, at least until solidification occurs during
309 fruiting body formation (see below). However, the cells are stably elongated by default, and thus
310 readily form liquid crystalline-like domains as in some animal tissues (Thutupalli et al., 2015).
311 The rapid relative movement of the cells, though, causes these to be only local and temporary.

312

313 **Solidification**

314 The generic-type fluid-to-solid transitions seen during development of the aggregative species
315 can productively be considered in relation to well-studied ones in animal embryogenesis. Animal
316 tissues during early stages of development, as noted above, behave in important ways like non-
317 living liquids. As development proceeds, however, some tissues undergo a transformation
318 where cell movements become constrained and the cellular mass behaves more like a solid
319 (Newman, 2019b). In these tissues, solidification may provide increased mechanical integrity,
320 and new morphological outcomes and constructional elements (e.g., exo- and endoskeletons)
321 arise with the physical properties of these materials. The most typical way solidification occurs is
322 by the deposition of stiff extracellular matrices (ECM), consisting of fibrous and nonfibrous

323 proteins such as collagen and elastin, covalently linked to, or complexed with
324 glycosaminoglycan-type polysaccharides. These ECMs can also become mineralized, as in
325 bone and tooth. More recently, “jamming,” a liquid-to-solid transition known from colloid physics
326 (Bi et al., 2011) has been shown to occur in liquid-state tissues as a result of increased cell-cell
327 adhesivity (Mongera et al., 2018).

328 In *D. discoideum*, cells are embedded in an ECM that once aggregation is complete defines
329 the boundaries of the aggregate. Aggregation in this and related species leads to the formation
330 of a migratory “slug” (see below), which once it reaches its final position, forms a fruiting body
331 by building up a stalk that takes cellular material away from the surface, and in which terminal
332 cell differentiation takes place. Membrane proteins involved in cell-cell adhesion are expressed
333 in a cell-type dependent fashion. Spores and stalk cells phase separate, in part, due to the
334 resulting differential adhesion, in agreement with the expected behavior of immiscible liquids
335 (e.g., water-oil mixtures), although other factors such as chemotaxis and differential cell motility
336 are also involved (see below) (Bretschneider et al., 2016; Raper, 1940).

337 During fruiting body elevation deposition of ECM, is required for the stiffening and
338 construction of the stalk (Palsson, 2008) (Dickinson et al., 2012). Solidification occurs unevenly
339 across the cellular mass. While the movement of cells in the stalk becomes constrained
340 because of the ECM, the remaining cells move upwards as the stalk continues to be built up
341 following the expected dynamics of solidifying non-living liquids. In Myxobacteria, deposition of a
342 stiff ECM appears to be the most important factor in aggregation, but the “solidification” of
343 maturing fruiting bodies may also involve jamming (Hu et al., 2012; Liu et al., 2019; Thutupalli et
344 al., 2015) see below).

345

346 **Differential loss of mass**

347 In animal morphogenesis, differential loss of mass can be achieved through programmed cell
348 death (e.g., apoptosis, autophagy and necrosis) where, in addition to acting as cue for signaling
349 pathways, it can also induce tissue reshaping by cell elimination or mobilization of mechanical
350 forces (Monier and Suzanne, 2015; Suzanne and Steller, 2013). In both Myxobacteria and
351 Dictyostelia, it has been suggested that programmed cell death may act as a mechanism for
352 nutrient release and recycling that can be employed for the remaining cells in the population as
353 source of energy and cellular materials (Boynton et al., 2013; Mesquita et al., 2017). However,
354 localized developmental lysis may also be relevant in mechanical reshaping multicellular
355 microbial masses. For example, localized cell death mobilizes mechanical forces that are
356 instructive for the generation of key features during development of *B. subtilis* biofilms (Asally et

357 al., 2012). In Myxobacteria, where most of the cells in the initial population undergo
358 developmental lysis, lysed cells may serve to strengthen the ECM (Hu et al., 2012). Specifically,
359 exopolysaccharides embedded in the ECM interact with extracellular DNA. As a consequence,
360 the ECM exhibits greater strength and stress resistance (Hu et al., 2012). While the origin of this
361 extracellular DNA remains unclear, it may be released by cells after lysis. In Myxobacteria and
362 Dictyostelia, peripheral rods and stalk cells, respectively, die after the stalk has been built up. In
363 both, cell death is a consequence of nutrition deprivation. In the dictyostelids, it shows
364 similarities as well as differences with the manner in which cell death is regulated in metazoan
365 tissues (Arnoult et al., 2001; Cornillon et al., 1994; de Chastellier and Ryter, 1977; Kawli et al.,
366 2002).

367 Additional generic effects can arise in cell masses from, e.g., synchronization of intracellular
368 biochemical oscillations. Some of these will be characterized below, after the roles of such
369 pivotal cellular functions in individual cell behavior are described.

370

371 **AGENT-LIKE BEHAVIORS IN MYXOBACTERIA AND DICTYOSTELIA**

372 Previous descriptions of the development of embryonic animal and plant tissues in terms of
373 material properties of multicellular assemblages have accounted for key morphological features
374 on the basis generic physical processes pertaining to these materials without invoking the idea
375 that individual cellular subunits of such materials act as autonomous agents in creating
376 multicellular forms and patterns (see, e.g., (Benítez et al., 2018; Newman, 2016). Although the
377 constituent cells in these generic accounts are assumed to carry out metabolic and synthetic
378 functions necessary to sustain life, to change their state (including polarity) in response to
379 external signals (Niklas et al., 2019), and (in the case of animal systems) locomote randomly,
380 the materials-based perspective does not involve formal sets of rules governing cellular
381 interactions of individually mobile cells. Similarly, as seen in the previous section, several
382 important aspects of Myxobacteria and Dictyostelia development can be explained by
383 considering them as generic materials, that is, considering the cell streams and masses as
384 generic liquid-like or solid-like materials.

385 However, attempts to computationally model aggregation of Myxobacteria and Dictyostelia
386 cells and the resulting multicellular masses based on generic mesoscale physics have found the
387 need to incorporate agent-like behaviors of the cells themselves into the models to capture the
388 relevant behaviors (Bahar et al., 2014; Fujimori et al., 2019; Marée and Hogeweg, 2001;
389 Thutupalli et al., 2015). (Following standard usage (Thorne et al., 2007) we define agents as
390 autonomous entities acting according to internal rules in a shared environment.) For biological

391 agents such as Myxobacteria and Dictyostelia cells these “rules” depend on intracellular
392 dynamics of molecules and pathways.

393 In biological development, agent-based phenomena pertain to the semi-autonomous
394 activities of individual cells or cells in transient associations with each other. This contrasts with
395 the collective effects governed by generic physical processes operating at the mesoscale.
396 Unlike nonliving systems, the subunits of tissues, aggregates, and presumptive aggregates are
397 living cells that are internally complex and chemically, mechanically, and electrically active and
398 potentially excitable. Cell dynamics can modulate the properties of biomaterials, making a liquid-
399 like animal tissue liquid-crystalline, for example, or a solid plant tissue locally expansible. When
400 cells act as individuals, however, alterations in their internal states can give them agent-like
401 properties when interacting with other such agents or features of the environment. The reality of
402 this distinction is illustrated by a recent study of neural crest migration where, exceptionally in
403 animal systems, cells navigate directionally through surrounding tissues in loose association
404 with each other. Consequently an agent-based modeling approach was deemed necessary
405 (Giniunaite et al., 2020a).

406 In certain cases, generic properties and agent-like effects mobilize the same intracellular
407 activities and processes. For instance, random cell movement, driven by actomyosin-based
408 contractile and protrusive activity, is essential to the liquid-like state of animal tissues. These
409 processes in individual amoeboid cells can also be mobilized for directional locomotion.
410 Similarly, concerted induction of cell polarity in animals and plants can impart anisotropy to the
411 respective tissues, changing their shapes and topology (Nance, 2014; Niklas et al., 2019). In
412 single amoeboid or bacterial cells, in contrast, polarity is essential in the sensing of chemical
413 and substrate gradients and directed navigation. Lastly, intracellular biochemical oscillation in
414 animal, amoebal, or bacterial cell collectives can attain synchrony, thereby causing it to behave
415 as a “morphogenetic field” in which cell states are coordinated at long distances across the
416 multicellular mass (Bhat et al., 2019 and references below).

417 As described above, multicellular systems can exhibit predictably similar morphological and
418 patterning outcomes as a result of mobilizing generic mesoscale physics. Agent-like behaviors,
419 however, are not generic in the same in sense, and their outcomes do not have the same kind
420 of shared inherency, since the rules that individual cells follow in relating to other cells and their
421 external environments are specific to each lineage and dependent on their respective
422 evolutionary histories. As mentioned above, and exemplified in the phenomena of directed
423 migration, regulated quiescence, and oscillation-based cell-cell communication, agent-like
424 behaviors of cells as distantly related as Dictyostelia and Myxobacteria can sometimes have

425 analogous morphological outcomes. This, combined with the generic effects with which they
426 interact in the development of multicellularity, contribute to the strikingly similar morphological
427 motifs in these disparate systems.

428

429 **Directed migration**

430 During animal embryogenesis, the displacements of cells relative to another can be largely
431 understood in terms of random movements analogous to the Brownian motion of the molecular
432 subunits of non-living liquid systems (Newman and Bhat, 2009). In Dictyostelia and
433 Myxobacteria, in contrast, cell trajectories deviate from the undirected motion of most animal
434 tissues due to the action of signaling and regulatory mechanisms. These bias the direction and
435 speed of cell movement in response to local cues in ways that may change as development
436 progresses. We suggest that some particularities of Dictyostelia and Myxobacteria observed at
437 the mesoscale (notwithstanding their shared liquid-like behaviors) derive from the distinct
438 mechanisms underlying directed cell migration in these two groups.

439 In Dictyostelia, cell movement occurs by amoeboid motion, which is driven by cytoplasmic
440 actomyosin-based contractile and protrusive activity just as in animal cells (Fukui, 2002). In
441 contrast to the generally random cell locomotion seen in animal tissues, however, Dictyostelia
442 exhibit both random movement and directed movement via chemotaxis, which can be thought of
443 as a biased random walk. Amoebae seek food by chemotaxis. Aggregation is also mediated by
444 chemotaxis, but to an aggregation pheromone (e.g., cAMP). Chemotaxis remains essential for
445 all subsequent developmental stages (Du et al., 2015). It dependent on both the physical
446 process of diffusion of the chemoattractant (which is not a generic tissue mechanism since it is
447 outside the cell mass) and agent-like behavior in response to the chemoattractant signaling at
448 the cellular level. Specifically, chemotaxis is a quantifiable outcome of directional pseudopod
449 extension (Chopra and Nanjundiah, 2013).

450 In *D. discoideum*, the response to the chemoattractant cyclic AMP (cAMP) involves an
451 oscillatory dynamics of excitation and adaptation (see below). The formation of streams with
452 high cellular density is facilitated by the collective movement of cells coordinated by chemotaxis
453 towards higher concentrations of cAMP. While cellular movements are most prominent at the
454 aggregation stages, extensive cell translocation still take place at later stages of the
455 development with chemotaxis biasing the individual movements. Cell movements remain
456 operational in the concerted movement of cells within a slug (Singer et al. (2019) but see
457 Hashimura et al. (2019). Finally, in **slugs and** maturing fruiting bodies, chemotaxis operates
458 jointly with differential adhesion to drive cell sorting (an authentically generic tissue process)

459 where it also provides the basis for fruiting body elongation (Matsukuma and Durston, 1979;
460 Schaap, 2011; Tan and Chiam, 2014).

461 In the case of Myxobacteria, where cells are rod-shaped, the presence of protein
462 complexes that promote motility defines a lagging and a leading pole (Guzzo et al., 2018). Cells
463 in transient contact with their neighbors move along their long axis in the direction of the leading
464 pole, with reversals in the direction of movement being a major agent-type behavior in
465 Myxobacteria motility. Reversals occur by switching the cellular polarity (i.e., the leading pole
466 turns into the lagging pole and vice-versa) and net cellular displacement is influenced by the
467 reversal frequency (Cotter et al., 2017). At the molecular level, reversals are controlled by the
468 Frz and MglAB intracellular oscillators (Guzzo et al., 2018; Igoshin et al., 2004). Directed
469 migration is favored during development by a reduction in the frequency of reversal that allows
470 cells to retain their direction and aggregate. This frequency reduction is stimulated by cell-cell
471 contacts, likely involving the exchange of intercellular signals, which become more frequent as
472 aggregation proceeds and cellular density increases (Cotter et al., 2017; Zhang et al., 2018). An
473 additional mechanism underlying directed migration in Myxobacteria is *stigmergy*, by which
474 individual cellular movement is biased by cues left behind by other cells (Gloag et al., 2016).
475 Specifically, while moving over solid surfaces, *M. xanthus* cells deposit slime material that forms
476 trails over which other cells travel preferentially.

477 In both Myxobacteria and Dictyostelia, the interplay between directed migration, an agent-
478 like behavior, and generic material properties highlights the need to consider them together in
479 accounting for development. In *D. discoideum*, cell sorting requires agent-like behaviors
480 (directed migration) and generic properties (differential adhesion) for its completion. In
481 Myxobacteria mesoscopic movement patterns are the result of the joint effect of the agent-like
482 behavior of directed migration and generic liquid-like behavior enabled by transient cell-cell
483 adhesion. In addition to these, the different phenomena observed along Myxobacteria life cycle
484 also require cellular alignment that may occur spontaneously as a generic property of rod-
485 shaped particles and cells (Janulevicius et al., 2015; Volfson et al., 2008).

486

487 **Cessation of movement and quiescence**

488 Development in *M. xanthus* and other myxobacteria starts as a response to starvation (Dworkin,
489 2007). Once it is sensed, ribosomes stall and the enzyme RelA increases the intracellular
490 concentration of the tetra- and pentaphosphate alarmones (p)ppGpp which, as in most bacteria,
491 induces the so-called stringent response (SR; (Boutte and Crosson, 2013; Cabello et al., 2017;
492 Chatterji and Ojha, 2001; Manoil and Kaiser, 1980a; Manoil and Kaiser, 1980b; Shimkets,

493 1999). As (p)ppGpp accumulates, proteases are synthesized and exported, leading to an
494 extracellular mixture of amino acids and peptides (A-signal), where it mediates a quorum-
495 sensing mechanism that enables a coordinated population-level response to starvation,
496 including specifying the minimal cell density required for initiation of development (Kuspa et al.,
497 1992). Myxobacteria respond to nutrient depletion via the SR, but also require high cell density
498 to initiate fruiting body and spore development. To effect this, in addition to conserved SR
499 components, Myxobacteria produce CgsA, which positively regulates (p)ppGpp and is in turn
500 positively regulated by it, and SocE, which suppresses and is suppressed by the production of
501 (p)ppGpp (Boutte and Crosson, 2013; Crawford and Shimkets, 2000a; Crawford and Shimkets,
502 2000b). Therefore, when A-signal rises to the concentration where it promotes aggregation
503 (Bretl and Kirby, 2016), which in non-aggregative bacteria would turn off the SR (since the A-
504 signal components serve as nutrients), the downregulation of SocE permits CgsA to keep
505 (p)ppGpp (which is required for spore formation) elevated during development.

506 A proteolytic cleavage product of CsgA serves as another extracellular signal which is
507 required for fruiting body development and sporulation (C-signal; (Giglio et al., 2015; Gronewold
508 and Kaiser, 2002). The specific mechanisms by which C-signal mediates intercellular
509 communication are not understood, but it appears to be involved in cell-to-cell adhesion and
510 coordination of cell movement during development (Sogaard-Andersen et al., 2003) and is a key
511 element enabling multicellular aggregation and cellular differentiation (Holmes et al., 2010;
512 Julien et al., 2000). In addition to A- and C-signaling, at least three other signals, termed B-, D-
513 and E-signal, mediate intercellular communication and coordination of individual cells during
514 development, but their specific mechanisms remain unclear (Bretl and Kirby, 2016; Kaiser,
515 2004).

516 The SR is largely conserved in bacteria where it typically mediates proliferative and
517 biosynthetic quiescence in response to nutrient depletion and other stresses. While it is
518 therefore likely to have been present in the unicellular ancestor of myxobacteria, the genetic
519 novelties represented by the intracellular CsgA-SocE circuits and the extracellular A-, B-, C-, D-
520 and E-signals co-opted this behavior to the transition to multicellularity. By making the SR cell
521 nonautonomous, these components and their interactions form a set of rules that enable cells of
522 *M. xanthus* to act as agents with respect to both cessation of movement and active signaling
523 (Arias Del Angel et al., 2017). As demonstrated in other myxobacteria such as
524 *Anaeromyxobacter dehalogenans*, and *Sorangium cellulosum*, it likely maintains aggregates
525 and promotes the differentiation of their constituent cells into quiescent spores and other cell
526 types (Huntley et al., 2014; Knauber et al., 2008).

527 Eukaryotic cells like those of Dictyostelium do not have a bacterial-type stringent response,
528 but they have their own conserved sensor of nutrient depletion, the enzyme AMP-dependent
529 protein kinase (AMPK). Among other effects, AMPK inhibits the energy utilization hub
530 mechanistic target of rapamycin complex-1 (mTORC1) under starvation conditions (Hardie,
531 2014). In animal systems AMPK plays developmental roles in, for example, inducing quiescence
532 in germline stem cells (GSCs) in the nematode *Caenorhabditis elegans*. In the absence of
533 AMPK, the GSCs overproliferate and lose their reproductive capacity, leading to sterility
534 (Kadekar and Roy, 2019). Significantly, in relation to the discussion above of the SR in
535 Myxobacteria quiescence, the function of AMPK in *C. elegans* development has been
536 reconfigured evolutionarily to be cell nonautonomous, with AMPK activity in somatic cells being
537 transmitted to GSCs via small RNAs (Kadekar and Roy, 2019). But the quiescence-inducing
538 role of AMPK is conserved across the eukaryotes, also appearing in plants and fungi (Guerinier
539 et al., 2013; Zhang and Cao, 2017).

540 In Dictyostelia, AMPK was found to regulate aggregate size and patterning, as well as cell
541 fate choice and stalk-spore case boundary formation in the fruiting body (Maurya et al., 2017).
542 Deletion of the gene specifying AMPK resulted in generation of numerous small-sized
543 aggregates (compared to wild type cell populations) that develop asynchronously to form few
544 fruiting bodies with small spore masses and long stalks. In contrast, when the gene is
545 overexpressed, cells form fruiting bodies with small stalks and large spore masses (Maurya et
546 al., 2017). Although AMPK itself functions cell autonomously, its regulation depends on
547 interaction with other cells, mediated by soluble factors. For example, the secreted inhibitor of
548 cell-cell adhesion Countin (Jang and Gomer, 2008) is upregulated in AMPK null cells, and
549 conditioned media collected from them cause wild-type cells to form smaller aggregates
550 (Maurya et al., 2017).

551 As with Myxobacteria, the starvation response triggers development at the expense of
552 growth. Jaiswal and coworkers have shown that although in Dictyostelium, mTORC1 function is
553 indeed inactivated via AMPK upon starvation, development is nonetheless initiated. These
554 investigators have identified of a class of essential starvation-upregulated, developmentally
555 associated signaling genes and downregulated growth genes (Jaiswal and Kimmel, 2019;
556 Jaiswal et al., 2019). Based on the earlier work of Maurya et al. (2017), downregulation of the
557 paracrine adhesion inhibitor Countin appears to be a component of this response, suggesting as
558 with Myxobacteria, a conserved starvation-sensing mechanism may have been recruited into a
559 mechanism of multicellular development by one or more factors that mediate communication
560 among agent-like cells.

561

562 **OSCILLATIONS AS A BASIS FOR BOTH GENERIC AND AGENT-TYPE BEHAVIORS**

563 Both Myxobacteria and Dictyostelia exhibit intracellular oscillations, which in the first case
564 mainly involves cell polarity and direction of motion reversals, and in the second, production of
565 chemoattractant molecules such as cAMP. Oscillations can mediate global effects if they come
566 into synchrony in established cell masses. This produces developmental fields in which the
567 constituent cells acquire a uniform state in a key modulator (e.g., the transcriptional coregulator
568 Hes1) and therefore are poised to respond to developmental signals in a coordinated fashion.
569 This occurs in animal systems, for example during the formation of somites, tandem blocks of
570 tissue along the central axis of vertebrates (Hubaud and Pourquié, 2014), and the digits of the
571 tetrapod limb (Bhat et al., 2019). The synchronization of oscillators can be considered a generic
572 physical effect since its physical basis is the same regardless of the underlying basis of the
573 oscillation.

574 But oscillations of individual cells can also provide component of agent-like behavior,
575 particularly in species that develop by aggregation. For example, they can permit cells to signal
576 one another over distances provided they are specifically receptive to periodic stimulation. The
577 myxobacterium *M. xanthus* exhibits a quasi-periodic reversal in the direction of motion. Reversal
578 in the gliding cells are achieved by dynamic cell polarity that switches direction by 180° (Zusman
579 et al., 2007). As noted above, regular reversals are driven by the relocalization of polarity and
580 motility proteins between the leading and lagging poles of the cells and allow for diverse
581 collective modes, such as rippling in nutrient-rich media (Mauriello et al., 2010; Shimkets and
582 Kaiser, 1982). Reversals also appear to be critical for complex collective behavior before and
583 during development (Blackhart and Zusman, 1985; Wu et al., 2009).

584 Indeed, it appears that reversal frequency in *M. xanthus* drives a phase transition from two-
585 dimensional flocking to one-dimensional streaming, therefore modulating the complex behaviors
586 that enable the robust formation of fruiting bodies (Thutupalli et al., 2015). Because the reversal
587 is coupled to intercellular signaling pathways (C-signal), this periodic switch may be
588 synchronized between different cells and favor development (Igoshin et al., 2004). A refractory
589 period, i.e., time lag in response to the environmental signal(s), in the molecular circuit
590 responsible for inducing the polarity reversal, has been proposed to underlie the rippling
591 dynamics of the bacterial sheet (Guzzo et al., 2018).

592 As in Myxobacteria, oscillations mediate collective behaviors in Dictyostelia, but they are
593 also the basis of agent-like behaviors in these social amoebae. Initially isolated cells of *D.*
594 *discoideum* aggregate by chemotactic movements in response to the release of periodic pulses

595 of cyclic AMP, which they also amplify and relay. Specifically, when stimulated with extracellular
596 cAMP, cells respond by synthesizing and secreting more cAMP. This results in non-dissipating
597 waves of cAMP which guide aggregation of individual amoeboid cells (Tomchik and Devreotes,
598 1981). The relay requires a refractory period, or else there could just be an explosive production
599 of cAMP with no local gradients to guide cells into aggregates. So, a nonconstant, ultimately
600 periodic, production of the chemoattractant by the dispersed cells is intrinsic to the patterning
601 process.

602 Since the cells in this organism start out as individuals, a key question in characterizing
603 their agent-like behavior is the relation of single cell oscillations to the global oscillations in the
604 organizing field of cells (Nanjundiah and Wurster, 1989). Isolated cells are capable of oscillating
605 (Sato et al., 1985), but it has been unclear whether such oscillations initiate the propagating
606 waves in the “excitable medium” constituted by the field of cells (Cohen and Robertson, 1971;
607 Durston, 1973). There are two physical possibilities. In the first, a set of oscillators (the
608 amoebae in this case) with identical period, but randomly distributed phases come into
609 synchrony or attain a spatiotemporal propagating mode through weak coupling, by a diffusible
610 chemical, for example (Garcia-Ojalvo et al., 2004; Kuramoto, 1984; Strogatz, 2003). The second
611 possibility is that cells only become oscillatory as a result of collective interactions, the global
612 behavior being an emergent process. Gregor et al. (2010) investigated these possibilities
613 experimentally and via mathematical modelling, and while they confirmed that isolated cells are
614 capable of oscillating, they concluded that the second possibility, what they term “dynamical
615 quorum sensing,” was the way that globally synchronized waves are generated in *Dictyostelium*.

616

617 **INTERPLAY OF GENERIC PROPERTIES AND AGENT EFFECTS**

618 As we have shown, aggregative multicellular systems can change their organizational states as
619 a result of the cell masses they form being shaped and reshaped by mesoscopic physical
620 effects, and also by lineage-specific, “custom-built” agent-like behaviors. A schematic
621 representing some of these factors and determinants is shown in Fig. 2. In some cases,
622 however, developmental transformations cannot be attributed to either category of effect alone
623 but can only be understood as outcomes of a combination of the two acting in concert. A newly
624 characterized example of this described by Hayakawa et al. (2020), in which an ordered, liquid-
625 crystalline-like field of polarized *D. discoideum* amoebae organizes by phase separation, from
626 populations of cells of a mutant strain incapable of chemotactic signaling via cAMP. This novel
627 patterning phenomenon, which has generic-type features, occurs by “contact following

628 locomotion,” a behavior whose agent-type role in the collective motion is supported by
629 simulations.

630 In the remainder of this section we will discuss two long-studied cases of such generic-
631 agential synergy: (i) the formation and migration of multicellular slugs in dictyostelids, and (ii)
632 formation of complex morphologies in fruiting bodies of both dictyostelids and myxobacterial
633 species.

634

635 **Slug formation in Dictyostelium**

636 When starvation drives *D. discoideum* into development the liquid-like streams that form
637 culminate in aggregation centers. The mature aggregates, slugs, migrate over the surface in
638 response to light and temperature gradients. Inside the slug, moving cells form smooth flow
639 patterns similar to those of individual particles in liquids (Vasiev and Weijer, 2003). The slug is a
640 long (~1 mm), thin (~50 μm) cylindrical mass with a well-defined anterior tip that directs its
641 movement. During aggregation and early slug formation presumptive stalk and spore cells are
642 sorted out along the anterior-posterior axis, and their relative positions become inverted in a
643 ‘reverse fountain’ manner as the fruiting body forms.

644 This process exhibits both generic mesoscopic properties but also agent-like behaviors of
645 the constituent cells. Odell and Bonner (1986), for example, used a continuum mechanics
646 model of viscous flow in which cells moved both longitudinally, in response to an anterior-
647 posterior cAMP gradient and transversely, in response to an unspecified gradient, to generate a
648 rotational movement that could generate a rolling flow. Jiang et al. (1998) employed a discrete
649 lattice model in which movement was determined by chemotaxis towards a center (the tip) and
650 energetics (cell-cell adhesion), and found that with the right balance of the two forces, a
651 reasonably correct pattern of sorting out resulted. Umeda and Inouye (2004) formulated a
652 continuum model of a viscoelastic fluid made up of heterogeneous actively moving points (cells)
653 that differed in various respects including their diffusive tendencies and abilities to offer
654 resistance, and obtained, in addition to sorting out, plausible equilibrium shapes for the slug.
655 Hogeweg, Marée, and coworkers combined agent-based and generic mechanisms –
656 chemotaxis to cyclic AMP, differential adhesion and pressure generation - to simulate the
657 aggregation of cells, the correct spatial distribution of cell type and their self-organization into a
658 fruiting body (Marée and Hogeweg, 2001; Marée, 2000; Marée et al., 2013; Savill and
659 Hogeweg, 1997). Trenchard (2019) has proposed a different agent-based mechanism for
660 sorting, one that depends on differences in speeds of movement and energetics.

661

662 **Fruiting body branching**

663 In contrast to *M. xanthus* and *D. discoideum* which exhibit branchless fruiting bodies, many of
664 the species in both of their lineages develop into branched structures (Schaap et al., 2006;
665 Yang and Higgs, 2014). In Dictyostelia, branches develop as the product of either budding or
666 from a secondary cellular mass generated through pinching off of the main cellular mass
667 (Schaap et al., 2006). These mechanisms can lead to different branching patterns in different
668 species, with in some cases arrays of secondary fruiting bodies arranged about a primary axis
669 of stalk cells (Gregg et al., 1996). In Myxobacteria, where evidence is more limited, branches
670 seems to develop exclusively by budding of the main cellular mass; pinching off has not been
671 reported in this group (Qualls et al., 1978). Also, regularity in the branch distribution, as
672 observed for whorl-developing fruiting bodies in some Dictyostelia species, is not obvious.

673 Cox and co-workers have carried out detailed studies on the genesis of the branching
674 pattern in fruiting bodies of the dictyostelid *Polysphondilium pallidum* (now *Heterostelium*
675 *pallidum*, Sheikh et al. (2018)), and their studies point to the integrated functioning of generic
676 and agent-like processes (reviewed in Bonner and Cox (1995). *P. pallidum*/*H. pallidum* fruiting
677 bodies are the result of secondary cellular masses being pinched off in regular intervals from the
678 primary cell mass as it moves upward as the main stalk is formed (Byrne and Cox, 1987). The
679 secondary masses turn into whorls of regularly spaced branches perpendicular to the main stalk
680 (McNally et al., 1987; McNally and Cox, 1988). As in *D. discoideum*, *P. pallidum*/*H. pallidum*
681 elongation involves chemotactic movements towards a cAMP gradient, the source of which is a
682 set of cells found at the tip of the cellular mass.

683 The mechanisms underlying pinching off of the secondary cellular masses remain
684 unknown. However, since this takes place before branching, the cellular mass may still retain its
685 liquid-like properties. Liquids may undergo pinch-off as a consequence of an imbalance of the
686 velocities of individual subunits across the mass. If the velocities are sufficiently large, the
687 adhesion forces will not be strong enough to keep the cellular subunits together and a (partial)
688 pinch-off would occur. As with slug locomotion, described above, chemotaxis could induce a
689 velocity gradient of the cells across the mass. Biased movement due to chemotaxis, along with
690 the oscillatory intracellular dynamics, may help to explain the observed regularity in the spacing
691 between the multiple secondary masses. This outcome, which is not trivially predicted from the
692 generic behavior of the liquid-like primary mass, may thus depend on agent-like behavior.

693 The secondary cellular mass remains attached to the stalk and rounds up as expected for a
694 liquid composed of homogeneously cohesive particles (McNally and Cox, 1988). Branches
695 developed from the secondary mass are regularly arranged across the plane perpendicular to

696 the main axis. The positions of the branches are proposed to be determined by a local
697 activation-long range inhibition effect like that described by Turing (1952), although the
698 components of this reaction-diffusion system have not been characterized (Cox et al., 1988).

699 The mechanism of branching itself is more problematic, since it is not an expected
700 morphology of liquid-like materials. Plant tissues, however, routinely undergo budding and
701 branching, an effect that has been attributed to the inherent properties of their material identity
702 as deformable solids (Benítez et al., 2018; Hernández-Hernández et al., 2012). These motifs
703 are independently recurrent developmental outcomes in all lineages of photosynthetic
704 eukaryotes, including the various polyphyletic algal clades and the monophyletic land plant
705 clade, the embryophytes (Hernández-Hernández et al., 2012). Since both Dictyostelia and
706 Myxobacteria undergo solidification via ECM deposition and possibly liquid-to-solid jamming in
707 portions of the multicellular mass after aggregation has been completed, this might allow the
708 multicellular masses to escape from the physical constraints imposed by the liquid-like behavior
709 and acquire the properties of deformable solids for which budding and branching are easily
710 achievable.

711 In addition to the transition from a liquid-like behavior to a solid one, a differential increase
712 of volume in the direction of the future branch is required to extrude from the main cellular mass
713 a secondary mass that will bud and finally turn into a mature branch. In plants, this is achieved
714 by localized cell proliferation in response to gradients of hormones (Benkova and Bielach, 2010;
715 Vermeer and Geldner, 2015). In Myxobacteria and Dictyostelia, development proceeds with
716 little, if any, cell division. One of two mechanisms, or a combination of them, might cause the
717 required increment in volume: further deposition of ECM or expansion of individual cell volume.
718 In either case, volume increase must occur in an irregular distribution over the mass, with foci of
719 hyperplasia specifying the sites where branches will develop further.

720 While some myxobacterial species also have branched fruiting bodies (see, e.g., Zhang et
721 al. (2003)), the lack of conventional chemotaxis (although see Taylor and Welch (2008) for a
722 chemotaxis-like effect in these organisms) and molecular networks for local activation-long
723 range inhibition may account for pinch-off and regular patterning in branching, respectively, not
724 being observed during fruiting morphogenesis in Myxobacteria. It should be noted that fruiting
725 bodies in these species grow vertically in a series of tiers, each involving the addition of a cell
726 monolayer. The rate of formation of new tiers is too rapid to be attributed to cell division, which
727 suggests that cells may be recruited from lower layers (Copenhagen et al., 2020; Curtis et al.,
728 2007). This mechanism for vertical growth is robust in the face of diverse mutations and
729 conditions, which suggest that it is an essential process in fruiting body morphogenesis (Curtis

730 et al., 2007). Since it has been reported that the deposition of tiers can be slightly asymmetrical
731 (Curtis et al., 2007), it is possible that branching in Myxobacteria arises from the amplification
732 and robust reinstatement of such asymmetries across generations.

733

734 **DISCUSSION**

735 Motivated by the parallelisms between the two major known lineages of multicellular
736 aggregative organisms: the prokaryotic myxobacteria and the eukaryotic dictyostelids, we have
737 reviewed the factors determining the main developmental events in these organisms. We
738 suggest that as a consequence of cell-cell contact during aggregation, the nascent multicellular
739 masses of each organism acquire liquid-like properties and thereby become subject to
740 morphogenetic processes characteristic of such materials. This allows them to be studied, and
741 in some respects explained, in terms of physical principles at the mesoscale. As expected from
742 the physical theory, the cell aggregates can exhibit streaming, rippling, and rounding-up
743 behaviors like those observed in non-living liquids.

744 While the molecules that mediate liquid-type properties in the two classes of organisms are
745 largely different, the physical processes mobilized at the multicellular scale are generic and in
746 that sense are the “same.” Furthermore, later in development cellular masses solidify and
747 behave as deformable solids, another category of material with nonliving counterparts with
748 generic properties. For such materials, branching is a predictable morphological outcome.

749 Although the behaviors in aggregating cells resemble those exhibited by non-living liquids,
750 mathematical and computational models have also needed to include agent-based behaviors in
751 addition to generic ones to achieve verisimilitude (Cotter et al., 2017; Fujimori et al., 2019;
752 Janulevicius et al., 2015; Marée and Hogeweg, 2001). Unlike the molecular subunits of
753 nonliving liquids, the cells constituting the multicellular masses can change and adapt their
754 behaviors in response to external cues through complex regulatory and signaling pathways. We
755 attribute the deviations of the dynamics and morphological outcomes of the multicellular masses
756 from generic physical predictions to the contribution of agent-like behaviors, e.g., directed
757 migration, regulated quiescence, oscillatory signal relay, reaction-diffusion coupling, of the cells
758 themselves. Cells of clonally developing multicellular organisms can also exhibit agent-like
759 behaviors (Christley et al., 2007; Giniunaite et al., 2020b; McLennan et al., 2020). While it is
760 difficult to quantify the relative contributions that each class of phenomena makes to the
761 respective developmental processes, considering the extent to which morphogenetic outcomes
762 are predictable from generic physical considerations we suggest that morphogenesis of

763 Myxobacteria and Dictyostelia is more dependent on agent-like behaviors than that of animals
764 or plants. This is almost certainly a function of their aggregative nature.

765 Because of the relative indifference of generic processes to molecular variation (adhesion,
766 for example, can be mediated by many different classes of proteins and glycans), the gene
767 products that first mediated the production of a form or structure in a species' earliest ancestors
768 need not be the same one that is active in its present members. Consequently, the gene
769 products that mobilize generic effects can differ widely in different classes of organisms (e.g.,
770 animals, plants, social amoebae and bacteria), and even in sister species, due to developmental
771 system drift (True and Haag, 2001). In contrast, generic processes are part of the physical
772 world, and therefore do not evolve per se, although the physics involved in a given lineage's
773 developmental routines can change over phylogeny (Newman, 2019a).

774 Many of the genes involved in generic processes in animal and plant lineages predated or
775 accompanied the emergence of multicellularity. In those lineages, morphogenesis and pattern
776 formation can be characterized in terms of the dynamical patterning modules (DPMs) that
777 mobilize specific physical forces and physicochemical effects to produce the respective
778 structural motifs (Newman, 2019b; Hernández-Hernández, 2012; Benítez et al., 2018). Similarly,
779 some gene products that shape dictyostelids and myxobacteria as multicellular materials were
780 carried over from single-celled ancestors, as were some gene products involved in agent
781 behaviors. However, as we have described with the *M. xanthus* stringent response suppressive
782 products CsgA and SocE, and the *D. discoideum* starvation-regulated paracrine factor Countin,
783 some agent-associated genes seem to be novelties of the social forms.

784 While DPMs are, by definition intrinsically multicellular, agents are intrinsically individual –
785 cellular, in the cases discussed here. Another important distinction is that agents are peculiar to
786 the biological world, even if they are artifactual (e.g., robots). Thus, in contrast to generic
787 materials, which have physically predictable macroscopic properties and behaviors, cellular
788 agents have no such constraints on their activities. The rules they follow in developmental
789 systems are as varied as cell behaviors (e.g., motility, secretion of ions, small and macro-
790 molecules, electrical, chemical, and mechanical excitability) and responses to
791 microenvironmental complexity permit.

792 Early comparisons between Myxobacteria and Dictyostelia noted that the morphological
793 outcomes of their respective developmental processes resembled one another to a remarkable
794 extent despite their independent origins, the evolutionary distance between them, and the lack
795 of gene-based homology in the relevant mechanisms in the two groups. Our attention to this
796 phenomenon was inspired by comparative analysis of the two lineages by Bonner (1982) and

797 Kaiser (1986). Both favored explanations based on convergent selection for adaptation to
798 similar ecological niches, with a focus on common developmental mechanisms such as cell
799 adhesion, communication and oscillations (Kaiser, 1986) and “developmental constraints” such
800 as that incurred by increased size (Bonner, 1982; Bonner, 2015). Based on the literature
801 reviewed here, we conclude that the similar developmental trajectories and outcomes of
802 Myxobacteria and Dictyostelia are more likely due to shared generic physical processes in
803 coordination with analogous agent-type behaviors than to convergent evolution under parallel
804 natural selection regimes. However, we acknowledge, in agreement with both Kaiser (1986) and
805 Bonner (2015), that ecology, in the form of exploitation or construction of suitable environmental
806 niches, is an essential factor in accounting for the establishment of these social phenotypes.
807 Our analysis extends beyond the molecular mechanisms considered by these earlier
808 investigators, to also include the physical nature of the multicellular masses. This approach is
809 based on experimental and theoretical advances made in material sciences, particularly as
810 applied to biological systems, in the intervening decades (see Forgacs and Newman (2005)),
811 and progress in agent-based concepts and models (Thorne et al., 2007).

812 Some authors have noted the tendency of aggregative multicellular organisms to exhibit a
813 narrower and simpler morphological diversity when compared to clonal organisms such as
814 animals and plants (Grosberg and Strathmann, 2007). A common explanation to this
815 observation is the emergence of genetic conflict arising between different cellular lineages being
816 incorporated into the same conglomerate during aggregation. Despite kin selection mechanisms
817 of “cheater” control (Travisano and Velicer, 2004), it is held that the impact of genetic conflict
818 could still be large enough to destabilize multicellular structure and impair the evolution of
819 further complexity. In clonal organisms, genetic conflict is thought to be avoided at every
820 generation by genetic bottlenecks that reduce genetic diversity to those mutations emerging as
821 consequence of DNA replication (Folse and Roughgarden, 2010). In his treatment of the
822 evolution of Dictyostelia, Bonner (1982) also suggested that selective regimens are dependent
823 on the scale on which they operate, and that size contributes to the differences in diversity
824 between Dictyostelia and Myxobacteria compared with plants and animals.

825 The physical framework addressed here provides an alternative to the multilevel selection
826 and scale-based accounts. As described above, despite the fact that animals, Dictyostelia and
827 Myxobacteria can all be conceptualized as non-living liquids, the weaker associations between
828 cells and surfaces in the social amoebae and bacteria lead to behaviors not observed in animals
829 (e.g., streaming) and the stronger, cytoskeletally linked attachments in animals mediate
830 behaviors (multilayering and lumen formation) not seen in the aggregative systems (Newman,

831 2019c). These differences are amplified by the fact that polarity (affecting, variously cell surface
832 or shape in the different systems) is much more transient in Dictyostelia and Myxobacteria than
833 in animals (Gómez-Santos et al., 2019; Manahan et al., 2004; Szadkowski et al., 2019),
834 undermining the persistence of complex organization in the former two groups.

835 An important implication of the perspective we have presented here is that physics-based
836 and agent-based approaches to understanding development are not simply alternative modeling
837 or computational strategies, but represent realities of complex biological systems that are
838 represented to various extents in different organismal lineages. Thus, the material nature of
839 multicellular systems and the inherent structural motifs entailed by the relevant physics
840 introduces a predictability to morphological evolution (Newman, 2016; Newman, 2019b). In
841 contrast, agent-type behaviors are more unconstrained and open-ended in their possibilities,
842 and their evolution could have led phylogenetic lineages that embody them (e.g., vertebrates,
843 which have the novelty of a neural crest (York and McCauley (2020)) in less predictable
844 directions.

845 Comparative analyses often rely on the study of homologous characters (i.e., those sharing
846 common ancestry) in order to disentangle phylogenetic relationships and hypothesize
847 evolutionary scenarios. These studies, mostly conducted in the population genetics framework
848 underlying the evolutionary Modern Synthesis, have provided important insights regarding the
849 processes of divergence of species as the product of selective pressures, genetic drift, mutation
850 and gene flow (Pigliucci and Müller, 2010). But (with some exceptions, see Abouheif and Wray
851 (2002)) they have generally neglected the role of development and, lacking a mechanistic view
852 of phenotypic innovation (Müller and Newman, 2005), are limited in the extent to which
853 homology can be assigned between characters in disparate groups (Müller, 2003; Müller, 2017).

854 Structures are considered homologous developmentally if they have the same form by virtue
855 of having the same generative processes. Here we have invoked a more general sense of this
856 concept, including in the notion of “sameness” generic physical mechanisms in addition to
857 genes. In this we are echoing the insights of the Soviet biologist N.I. Vavilov, who in his classic
858 paper “The law of homologous series in variation” wrote, “[g]enetical studies of the last decades
859 have proved even the divisibility of the minutest morphological and physiological units in
860 systematics...and established that, although outwardly similar, they can be different
861 genotypically” (p. 48), and that “the great majority of varietal characters, not only within the limits
862 of single genera and families but even in distant families, are homologous from a morphological
863 point of view” (p 82) (Vavilov, 1922). We suggest that our broader concept of homology can help
864 resolve enigmas of biological similarity across phylogenetic distances. Knowledge of molecular

865 and cellular determinants of material identity and agent-like behaviors, in concert with suitable
866 mathematical and computational models of these causally hybrid, multiscale systems (e.g.,
867 (Camley and Rappel, 2017; Cotter et al., 2017)), could ultimately provide a compelling and
868 testable account of these morphological affinities.

869

870

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872

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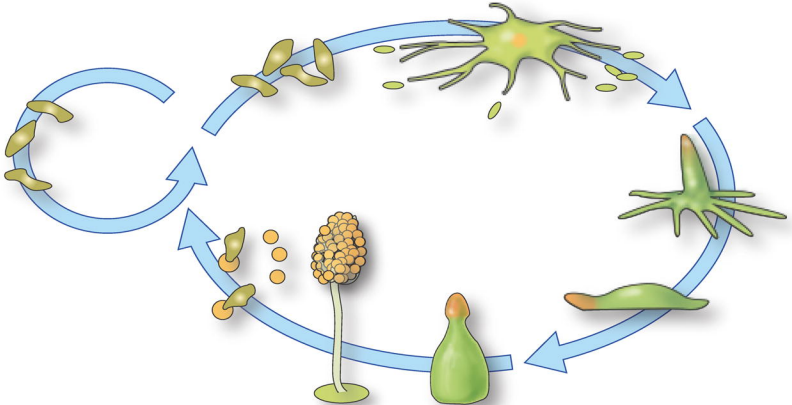
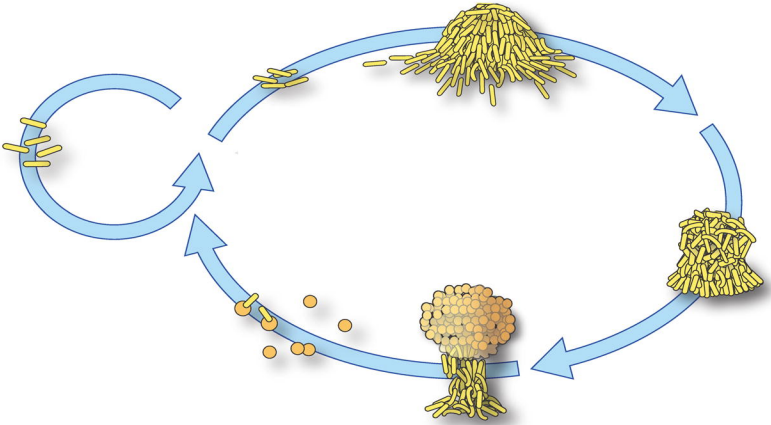
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Figure legends

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Figure 1. (Upper panel) Life cycle of *Myxobacteria xanthus*, a representative multicellular myxobacterium. The circle on the left represents the proliferative mode that occurs in a nutrient-replete setting. The oval on the right shows the sequence of stages initiated under conditions of starvation: clockwise, from top left, aggregation, mound formation, fruiting body formation and spore differentiation. Spores can be dispersed and may germinate as single vegetative cells under nutrient-rich conditions. (Lower panel) Life cycle of *Dictyostelium discoideum*, a representative dictyostelid. The circle on the left represents the proliferative mode that occurs in a nutrient-replete setting. The oval on the right shows the sequence of stages initiated under conditions of starvation (clockwise, from top left: starved amoebae, developing aggregation, late aggregations, migrating slug, developing fruiting body, finished fruiting body with spore mass supported by an erect stalk, amoebae emerging from spores after dispersal).

Figure 2. Schematic representation of (left, top) a selection of generic physical effects and one of their underlying mediators (cell-cell adhesion), and (left, bottom) a selection of agent-like effects, all of which pertain to aggregative multicellular organisms such as myxobacteria and dictyostelids. Some individual cell behaviors like biochemical or polarity oscillation can, when they operate in the multicellular context, can mediate global generic effects, like morphogenetic fields in which cell state is coordinated over large distances. Generic processes can lead to convergent morphologies since they employ the same mesoscale physics despite genetic divergence. Agent-based processes can lead to lineage-specific behaviors and morphological motifs, but also convergent or parallel ones if they act in analogous fashions. See main text for additional examples of generic and agent effects, and descriptions of their morphogenetic roles.



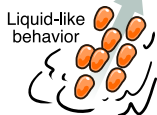
Generic properties



Adhesion



Differential loss of mass



Liquid-like behavior

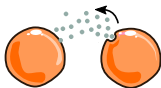


Solidification

Agent-like properties



Directed migration



Active signaling



Cessation of movement



Oscillation

Convergent traits and behaviors

Synchronization
Field formation

Lineage-specific
traits and behaviors