**Inherency and agency in the origin and evolution of biological functions**

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Although classically discussed in terms drawn from the sciences of non-living systems, biological function in recent decades has been considered in relation to organismal capability and purpose. Bringing two phenomena generally neglected in evolutionary theory – inherency and agency – to bear on questions of function leads to a rejection of the adaptationist “selected effects” notion of biological function. I review work showing that organisms like the placozoans can thrive with almost no functional embellishments beyond those of their constituent cells and physical properties of their simple tissues. I also discuss work showing that individual tissue cells and their artificial aggregates exhibit agential behaviours that are unprecedented in the histories of their respective lineages. I review findings on the unique metazoan mechanism of developmental gene expression that has recruited, during evolution, inherent ancestral cellular functionalities into specialized cell types and organs of the different animal groups. I conclude that most essential functions in animal species are inherent to the cells from which they evolved, not selected effects, and that many of the others are optional “add-ons,” a status inimical to fitness-based models of evolution positing that traits emerge from stringent cycles of selection to meet external challenges.

ADDITIONAL KEYWORDS: adaptationism – biobots – causal role – cell types – fitness – function-appropriation engine – niche construction – placozoans – selected effects.

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INTRODUCTION

The problem of biological form, specifically its origin and reshaping, was a major impulse to developing theories of evolution once forms were recognized to differ from one stratigraphic layer to another in fossil deposits. But the central issue in all such theories is not form itself, but biological function. This is because the uses to which morphological attributes are put, not their details and arrangements, have defined the lives of the respective organisms. Physiology, not anatomy, was the basis for generating now-extinct animals and plants, and its eventual inadequacy is the reason they no longer exist. The functions associated with preserved forms, e.g., flexure of the limbs or vision in the orbits, are typically based on components – muscles and eyes – that are not retrievable. Many functions cannot be discerned from fossilized remains, or even from DNA remnants: warm-bloodedness, vocalization, and cognition, for example. But inferences about them can be critical in evolutionary interpretations. Rationalizing or explaining biological functions underlies all theories of evolution, whether Lamarckian or Darwinian and all that have succeeded them.

Darwin’s theory of natural selection, the most widely accepted account of evolutionary change, depends on the concept of *fitness* – the capacity or disposition to leave progeny in the next generation – to account for functions. This is based on the recognition that there is heritable (gene-based) variability in the expression of function-related traits. Over time, an incipient functional trait turns into a full-fledged one, or an existing one is transformed into another. Functions, like other traits, are assumed to be typically polygenic, the result of cooperative interaction of many genes. To analyse how a function becomes established in a population that previously lacked it, an idealization, Ronald Fisher’s “infinitesimal model,” is often applied (Fisher, 1918). In this model, each locus is held to make a small enough contribution to the overall genetic variance that the latter can be presumed to remain constant as natural selection on different alleles takes place.

The gradualism implied by the infinitesimal model precludes a role for “genes of large effect.” This case was later reinforced by Fisher in his “geometrical model” (Fisher, 1930), where he focused more explicitly on the intra-organismal compatibility of genetic novelties – what would now be termed “biocomplexity.” Fisher argued that the more independent dimensions of variation the phenotype has, the more difficult it would be to improve it (in the sense of increasing its fitness) by random genetic changes. Thus, the smaller its effect the more likely a change would be beneficial. This model remains a tenet of the Darwinian framework (although cf. Nelson *et al.* (2013)). Seventy years after it was proposed, Orr calculated the rate at which fitness increases during adaptation in organisms with varying complexity. Complexity was defined, following Fisher, as the dimensionality of the space of the individual’s independent “parts” or traits. He concluded that both the probability of fixation of a beneficial mutation, and the fitness gain conferred by it, decrease with organismal complexity (Orr, 2000).

Although it is now recognized that large-effect genes can indeed contribute to evolutionary change (Stoltzfus & Yampolsky, 2009) – the spines of the stickleback (Indjeian *et al.* (2016) and the head crest of rock pigeons (Shapiro *et al.* (2013) being well-known examples – Darwinian theory, even in its various expanded and extended versions, is committed to gradualism. Darwin himself rejected the ideas that the “sports” or “monstrosities” seen by animal and plant breeders could be sources of evolutionary change (Darwin, 1868). Similarly, the speculation by the geneticist Richard Goldschmidt (Goldschmidt, 1940) that the anomalous organisms seen in laboratory settings were prototypes of natural “hopeful monsters” that could provide the basis of new phylogenetic lineages was dismissed by the formulators of the Darwinian population genetics-based Modern Synthesis (Mayr, 1982). The reason for adhering to gradualism is simple: slow accretion of imperceptibly small changes and their culling by natural selection is Darwin’s specific theory of the origin of biological complexity: “Darwin’s dangerous idea” (Dennett, 1995 – see also the discussion in Gould (2002): 139–141). Formation of a complex structure by abrupt means requires a different explanation, one based on development. Selection could follow after the fact, influencing the biogeographical distribution of the variants, but it would not have a role in fashioning them. If this “phenotype first” scenario (West-Eberhard, 2003) has been a general trend in the origin of biological diversity, or was so at early stages of phylotypic diversification, the importance of natural selection as a creative force would be greatly diminished.

We have proposed that multicellular aggregates, particularly those constituting developing animals and plants, have physical propensities that made the acquisition of certain morphological motifs all-but-inevitable (Newman *et al.*, 2006; Newman & Niklas, 2018). In the case of animals, these include, tissue layers, lumens, segments, and appendages (Newman, 2019), and in plants, leaves, buds, and branches (Benítez *et al.*, 2018). That is, the material properties of these systems inhabit “morphospaces,” domains of anatomical possibility that are both copious and constrained (McGhee, 2011). While these are not fully occupied at the inception of new kinds of multicellular matter, they can become more so over time due to changes in the external environment or genes that induce abrupt transitions in the respective developmental systems. Some mutations of pre-existing genes have non-linear consequences, making them of “large effect.” In other cases, novel, sometimes unprecedented, genes were coincident with the founding of certain lineages (the diploblastic animals, for example), and, by mobilizing previously unused physical effects, opened new morphological potentials.

To reiterate: if morphological novelties arose by means other than cycles of gradual change, evolution of form cannot mainly be a question of fitness and relative advantage, but rather of development and its transformations. Natural selection would have inevitably influenced the ecological prevalence and distribution of morphological variants, but not as an agent of their innovation.

The evolution of physiological functions seems to be a different matter. Unlike anatomical traits such as fins, wings, scales, and eyes, which can be dispensable options facilitating exploration and occupation of novel niches, functions like the circulation of blood, exchange of gases, and the excretion of toxins, are typically essential in sustaining life. There are exceptions in either direction: some anatomical units – the heart, lungs, and kidneys, for example – are necessary for survival, but only because they harbour essential functions. Thermogenesis is a dispensable function, but only in the evolutionary sense. For lineages committed to it – birds and mammals among the jawed vertebrates – it is essential.

All functions are based on complex multigene pathways. Unlike anatomical innovation, which can occur when large-effect genes elicit unused morphogenetic inherencies or (by expanding the developmental system’s morphospace) mobilize new ones, functions do not seem to be something that could arise other than by gradual means.

In what follows however, I suggest, counterintuitively, that most physiological functions appeared in animal lineages abruptly and essentially ready-made. The basis for this is also a set of inherencies: in some cases (including the morphologically simplest of the animals) this is related to the physics of chemically and mechanically excitable materials. But in more complex animals, new cell types and functions are recruited during evolution from the life-sustaining activities of these organisms’ single-celled ancestors. Recent evidence indicates that this appropriation of inherent functionalities occurs by a cell nuclear “differentiation engine” unique to the metazoans, the recognition of which must inevitably change the way we understand the origination and evolution of biological function.

The structure of the rest of this paper is as follows: first I will briefly summarize some widely discussed approaches to the definition of biological function. Following that, I will discuss the example of the placozoans, a morphologically very simple, early diverged animal which, lacking major components of the metazoan differentiation engine, employs a meagre set of cell types and functionalities along with a set of exotic, though explicable physical effects to sustain its existence. In the same section I also describe an even simpler, experimentally produced model, the “biobot” multicellular spheroids of Levin, Bongard and co-workers (Blackiston *et al.*, 2021; Kriegman *et al.*, 2021), to illustrate the capacity of multicellular forms to appropriate functionalities of their constituent cells. Even in the absence of a selective-adaptive evolutionary history, these constructed entities invent and perform novel activities and manifest unprecedented enablements. In the section following that, I describe the metazoan chromatin-based apparatus that generates specialized cell types in more complex forms, and I situate it in the evolutionary history of the animals, noting its deficient instantiation in the placozoans and its enhancements in the vertebrates. In the final section, I discuss the implications of inherency, optionality, and “generative entrenchment” (in the sense of Wimsatt, 2015), in a revised conception of biological function.

THEORETICAL ACCOUNTS OF BIOLOGICAL FUNCTIONS

Attempts to conceptualize biological function have engendered disparate views in the philosophy of biology (Wouters, 2005). Some (“causal role”) theorists (e.g. (Cummins, 1975; Wright, 1976) have focused on function in relation to the role a process (the heart’s pumping blood is the one always given, but wings and flight are also frequently cited) plays in a presently existing organism. This position was explicitly staked out in contradistinction to those of analysts who focused more strictly on the necessity or sufficiency of a trait to produce an identified effect (Nagel, 1961; Hempel, 1965). Bringing purpose into the notion of function thereby invoked a view articulated by William Harvey in the 17th century (Harvey & Keynes, 1928; see discussion in Allen & Neal, 2020). According to Cummins’s theory of functions, for example (Cummins, 1975; see also Buller, 1998), an item has a function only relative to a capacity of a system, where its function emerges from a functional analysis of the capacity.

Some causal role theorists employ specific notions of biological organization in attributing functions to specific traits. This builds on Maturana and Varela’s concept of autopoiesis (Maturana & Varela, 1980), an attempt to provide a constitutive (rather than merely heuristic) account of the self-organizing, self-maintaining nature of living systems – Kant’s “natural purpose” (Kant, 1790; trans. 1966). The function of a trait in these analyses is defined by the role it plays within an organized system in contributing not only to the system as a whole but to its own persistence (Schlosser, 2004; McLaughlin, 2000; Saborido *et al.*, 2011). Thus, the heart contributes to the maintenance of the entire organism by facilitating the circulation of oxygen and nutrients, and it also functions to promote its own preservation, since its cells require the delivery of oxygen and nutrients for their survival. “Organizational closure” (Moreno & Mossio, 2015), a conceptually more sophisticated extension of autopoiesis, holds that organisms realize an emergent regime of causation such that its constituents, each of them constraining the operation of the others, collectively maintain themselves via a mutual dependence (see also Artiga & Martínez, 2016).

Causal role approaches infer the function of a trait from its present, or in certain cases, ancestral, role in an organism’s activities. Although it is always acknowledged that traits are products of evolution, these theories do not explicitly consider this in ascribing functions. Complementary to these approaches, however, are etiological perspectives identifying functions not only by their roles in sustaining organismal integrity (also exhibited by artificial hearts or a plaster cast for a broken bone) but also in their having arisen and served the corresponding role (or an earlier version of it) in the organism’s ancestors. These are the only “proper functions” according to Ruth Millikan (Millikan, 1989; see also Thomas, 2017). The heart, for example, pumps blood but also makes sounds. Only the first is a proper function in this view. Since natural selection is the usual explanation for how organs and their functions arise, proper functions were synonymized by Karen Neander to “selected effects” (Neander, 1991), a framing that has become standard.

Buller (1998) characterized weak and strong etiological theories of function, both of which he asserted to depend on a causal role concept like that of Cummins. In the weak form, a trait has a function in causing an effect if it contributed to the fitness of an organism’s ancestors by producing the same or a similar effect, and is hereditary. These criteria for function, however, do not disqualify cases in which traits are inherited, but subvert, rather than support, the integrity of an organism. (The segregation distorter gene of *Drosophila melanogaster* is such a case.) It also fails to reject cases where a trait is inevitably inherited along with a functional one but does not perform a useful function. (The extra weight of a bear’s fur, in contrast to its capacity to provide warmth, is an example of this, as is the odour of excreta as a sensory attractant for predators.)

Motivated by such arguments (see Godfrey-Smith, 1994), Buller (1998) introduced a strong form of the etiological function concept. According to this version, there is selection for a given trait due to its producing the effect in question, but an additional stipulation is that there must have been heritable variation in the trait at some point in the direct evolutionary history of the lineage. Further, the bearers of the current variant must have been more fit than cohorts with different variants precisely because of the produced effect.

As the term “proper function” implies, some theorists disqualify non-selected traits as conceptually coherent examples of functions. By this criterion, physical effects, such as the gravity-driven localization of developmental determinants in the frog egg, or surface tension-driven rounding up of the blastula of an early-stage zebrafish embryo (Newman & Comper, 1990), do not count as functions, even though they are inherited and directly responsible for essential outcomes.

Some features of the individual cells that constitute organisms have clearly purposeful operations or functionalities of their own. In addition to organelles such as mitochondria and cilia with well-defined functions and generally accepted (for mitochondria) and debated (for cilia) origins, the cells of multicellular organisms can also produce their bounding membranes and proteins and grow in mass and divide (sequestering their genetic material) when they reach a critical size. Since performance of these tasks in all known cells is all-or-none, any assertion that they were “selected” to perform these activities is just a matter of lack of alternativies. Since the elaborate biochemical pathways that support these basic functions in different environments and lineages were accumulated progressively and have a modular organization, they might qualify as proper functions, but the bases of their origination and refinement are obscure. They could have arisen (as proposed for the origin of the genetic code) by “collective, but non-Darwinian, mechanisms likely to be present in early communal life” (Vetsigian *et al.*, 2006).

Here, in theorizing the evolutionary emergence of differentiated cell types and the functions of associated organs, I take as my domain of reference the non-metazoan holozoans, the closest relatives of the animals (Sebé-Pedrós *et al.*, 2017). The main idea derives from recent findings that the appearance of the animals was accompanied by a gene regulatory apparatus unlike that of other cells, including direct ancestors (Newman, 2020a). This system consists of protein and nucleic acid (DNA and RNA)-containing chromatin hubs poised to recruit the multigene pathways of single-cell functionalities (“protofunctions”) into subpopulations of embryonic cells. It thus converts them into specialized cell types.

Because protofunctions, having evolved in ancestral holozoans or earlier organisms, are present in the embryonic cells of the various animal species, each of them can potentially be elicited by various effectors. External stress, for example, has plausibly been proposed to be among these effectors (Love & Wagner, 2022). My concern here, however, is with inherent functionalities and processes that provide the raw material for the induction of new cell types.

There are in fact viable animals with no organs and as few as six differentiated cell types (Smith *et al.*, 2014). These did not disappear when more functionally sophisticated forms arose. Aggregates of one or two kinds of embryonic cells can also flourish in and navigate *in vitro* conditions (Blackiston *et al.*, 2021; Kriegman *et al.*, 2021). These functionally and morphologically simple forms exhibit agential behaviours which clearly derive from the agency of the cells that compose them. (Such agential behaviour is not unique to animal or holozoan cells, but pertains to all forms of cellular life, even bacteria: (Arias Del Angel *et al.*, 2020; Levin *et al.*, 2021).) The implication of these two examples is that multicellular entities consisting of animal cells can make their way in the world without elaborate structural and functional specialization, which are thus not needed for survival.

The next section will describe some of these natural and experimentally produced simple metazoan systems, placozoa and (briefly) marine sponges on the one hand, and the recently described “biobots” on the other. From these we can infer a “ground state” of animal identity, on which morphological development was played out during evolution by occupying new regions of the morphospace of metazoan multicellular matter (Newman, 2019). In addition, by mobilizing unusual but physically explicable self-organizational effects, or appropriating pre-existing activities of constituent cells, novel functional attributes were introduced, “add-ons” that enabled new forms of living activities and agency.

ANIMALS WITHOUT ORGANS OR (MANY) CELL TYPES

While there is an essential core set of functionalities in all eukaryotic cells, the cellular composition of placozoans, the morphologically simplest of the animals, gives no support for the ideas that specialized physiological functions beyond those exhibited by ancestral cells are necessary to support multicellular life. *Trichoplax adhaerens*, a millimetre-scale organism with a fluid-containing interior bounded by two epithelium-like layers, has six (Smith *et al.*, 2014) (or, by recent estimates, as many as nine Schierwater *et al.* (2021)), somatic cell tyes, which play mainly structural roles but also mediate a limited number of functional activities based on enhancements of pre-existing cell capabilities (Yanez-Guerra *et al.*, 2022).

The dorsal (upper surface) and ventral (substrate-facing surface) epithelia of *Trichoplax* differ in some surface elaborations and cytoplasmic inclusions, but both contain cilia – externalized counterparts of intracellular structures found in all eukaryotes. Both the dorsal and ventral epithelia lack a basal lamina, a stiff, planar extracellular matrix (Smith *et al.*, 2014). All more complex animals produce such materials, which anchor epithelial sheets. Without this mechanical constraint, the placozoan epithelia behave as “excitable media” (also referred to as “active matter”), exhibiting physical effects not seen in any non-living or even (thus far) other biological system. The cells of the dorsal epithelium, for example, undergo sporadic and coordinated cycles of contraction that can be manifested at the tissue level in propagating mechanical waves that are the fastest in any known animal (Armon *et al.*, 2018). These movements, which function in the asexual division of the entire animal, are based on a subcellular actomyosin contractile apparatus like that of nonmuscle cells in other animals and, more relevantly, holozoan ancestors (Thattai, 2019). However, due to its operating under “no load conditions,” the epithelium behaves in this highly exotic fashion, mediating the animal’s highly plastic and dynamic morphology.

The ventral epithelium is similarly mechanically unconstrained but is more delicate and deformable than the dorsal layer. It also exhibits travelling waves of elastic displacement, but these are highly influenced by the movement of its cilia, which undergo a collective self-organizing behaviour with similarities to the flocking of birds or schooling of fish (Bull *et al.*, 2021a; Bull *et al.*, 2021b). This previously undescribed set of complex mechanical interactions is the basis of the ability of the placozoan to glide along its substratum.

Ciliated epithelial cells constitute 72% of the total cell population of *T. adhaerens* (Smith *et al.*, 2014). Interspersed among them in and near the ventral layer are gland cells (part of a cell population constituting 3% of the total) producing TaElp, an endomorphin-type peptide that regulates ciliary beating (Senatore *et al.*, 2017). The ventral epithelial cells, which also contain microvilli, are intermixed with a non-ciliated population termed lipophil cells (about 11% of the cellular population: (Smith *et al.*, 2014). These secrete proteolytic and lipolytic enzymes of paneukaryotic (i.e., premetazoan) origin (Sebé-Pedrós *et al.*, 2018), enabling this epithelium to serve as an external digestive system for the cyanobacteria and microalgae on which the organism feeds.

Some gland cells appear to provide neurosecretory effectors of the digestive function. These cells contain the SNARE secretory complex (syntaxin1, synaptobrevin, and SNAP-25) and some express the synaptic vesicle protein synapsin (Smith *et al.*, 2014), all of which have premetazoan holozoan roots (Göhde *et al.*, 2021). Although the organism contains no neurons, FMRFamide, a neuropeptide found in molluscs, is secreted by a subset of its cells. While the gene for this peptide has thus far not been identified in a non-metazoan holozoan, a portion of the nesfatin-1 neuropeptide gene, which has pre-metazoan holozoan origins, is present in the *T. adhaerens* genome (Yanez-Guerra *et al.*, 2022). The full gene may have been lost in the evolution of Placozoa. The lack of co-expression of the genes involved in synaptic and neuronal functions in peptidergic cell types indicates that none of the gland cells have a synaptic neuron-like identity (Sebé-Pedrós *et al.*, 2018). The possible reason for the lack of counterparts in this organism of functionally sophisticated cell types like those of eumetazoans is discussed below.

Fibre cells (about 4% of the cell population) are a phagocytic cell type arranged in a single sheet-like layer in the interior of *T. adhaerens* (Smith *et al.*, 2014). They are multiply branched and interconnected by processes with open ends, making them syncytial. Fibre cells also contact the inner surfaces of both epithelial layers, indicating a structural role in maintaining organismal integrity (Mayorova *et al.*, 2021).

Finally, at the periphery of the organism beneath the dorsal epithelium and in contact with fibre cell processes, is a rare (~0.2%) population of “crystal cells” with calcium carbonate inclusions. These appear to be statocytes, i.e., gravity sensors (Mayorova *et al.*, 2018).

To summarize, placozoans, despite their morphological simplicity, exhibit both anatomical features and physiological functions. The first consist of encompassing epithelia and an internal fibrous supporting network. The second comprise sporadic and wave-like mechanical excitations of the dorsal epithelium leading to whole-organism shape changes and scission, locomotion, digestion, and gravity sensing.

The mechanical excitability of the dorsal and ventral ectoderms mediates properties of these tissues that unquestionably serve survival functions in placozoans. But as inherent material properties they are not “proper functions” as defined above. Further, the appropriated functionalities represented in the secretory and sensory cells are inherent to single cell existence. Therefore, neither type of placozoan function is a selected effect. And while its species embody the minimal known anatomical and physiological arrangement for sustaining animal life, Placozoa exemplifies an evolutionary dead end. A fitness-driven model of adaptive evolution would suggest there should be other organisms phyletically related to placozoans with more cell types and other embellishments, such as observed for phyla like molluscs or chordates. But all known members of this phylum are morphologically and functionally similar.

In addition to lacking a basal lamina, placozoans as so-called basal metazoans lack the planar polarity pathway (Newman, 2019). This prevents them from implementing morphogenetic processes such as tissue elongation, reshaping, and folding that are involved in the generation of organs and organ-like arrangement of cell masses in the more complex eumetazoans (all animals other than placozoans, and poriferans – marine sponges). Furthermore, in contrast even to sponges, placozoans lack important components of the Notch signalling pathway (Gazave *et al.*, 2009). This pathway mediates lateral inhibition and other aspects of developmental pattern formation, and thus placozoans may be less effective in arranging differentiated cells.

Most important for the discussion here, however, placozoans (again in contrast to poriferans) do not utilize enhancers for developmental gene regulation (Sebé-Pedrós *et al.*, 2018). Enhancers are DNA sequences distant from a gene’s proximal (adjacent) promoter, which unlike promoters (even distal ones) can increase expression dramatically. The enhancer-based system, discussed in the next section and termed the metazoan “function-appropriation engine” (FAE), permits the coordinate expression not only of a few individual genes via gene-proximal promoters (as in the placozoan cell types) but of more complex pathways related to ancestral unicellular activities. Employing distal promoters and enhancers in conjunction with pioneer and lineage-determining transcription factors (see below), the expression hubs of the engine partition cellular functionalities into complex, cooperating cell types.

It is notable that the marine sponge *Spongilla lacrustris*, which like other poriferans does contain the FAE, has 18 cell types, many with lineage and functional relationships to one another (Musser *et al.*, 2021). Examples include the family of peptidocytes which share a role in digestion. They include choanocytes, which form chambers housing this activity, and apopylar cells, which constitute the pores of the chambers. The mesohyl, an acellular extracellular matrix compartment of the sponge, is the site of another subclass of these cells, the myopeptidocytes. These have long projections that contact each other and other cells, including choanocytes. They express actomyosin-based contractility modules and associated proteins that may control contraction of the mesohyl. Notwithstanding the simplicity (compared with eumetazoans) of the poriferan body plan, these cell functions and relationships represent a large step beyond their counterparts in Placozoa.

If the capacity to enlist interrelated inherencies can make the difference between a placozoan’s generation of 6-9 functionally simple cell types and a sponge’s generation of 18 more elaborate, interactive ones, what might we learn from multicellular entities unable to turn any of the functionalities of their constituent cells into cell types? A recent set of experiments gives us a window into a world of organisms with (almost) no functions.

In one set of experiments, Levin, Bongard and co-workers (Blackiston *et al.*, 2021) isolated fragments of embryonic epithelium from the top surface (“animal cap”) of frog embryos, allowed them to round up into balls of several thousand cells (comparable in size to a placozoan), and observed their behaviour in culture. These spheroids spontaneously organized into an inner core of epithelioid (i.e., directly contacting) cells covered by a layer of similar cells with multiple exterior cilia. Locomotion of these entities (termed “biobots” by the authors) occurred by the concerted beating of cilia (as in placozoans). They moved through mazes, often by navigating long channels without reversals, and exhibited and switched among different patterns of free motion. They also showed emergent group activities in which the efficiency of one of the spontaneous behaviours, the active piling up of inert particles, was enhanced in collectives of spheroids.

In another set of experiments (Kriegman *et al.*, 2021), the animal caps were dissociated and permitted to re-associate into aggregates, which rounded up into spheroids, as with the fragments. In this study the culture environment was seeded with dissociated cells rather than inert particles, and the spheroids were observed to assemble these into piles, which aggregated and formed additional spheroids, implementing a novel form of reproduction.

Although the biobots are derived from a vertebrate organism, i.e., one that embodies the cell differentiation-generating FAE, they do not employ it during these experiments, since no development takes place. The biobots behave as autonomous organisms although they lack tissue-level morphological and functional elaborations other than the cilia of the embryonic cells. Surprisingly from the standpoint of adaptationist models, they exhibited functional capabilities that were unrelated to anything in the evolutionary history of the frogs from which they were derived. Their apparent agency was unquestionably due to their being composed of living cells, but it was both different from single-cell agency and unprecedented.

Such experiments cannot provide any direct insights into the evolution of animal function, but they inspire speculations about the transition from non-metazoan holozoans to the (unknown) immediate ancestors of the placozoans. Assuming those hypothetical forms similarly lacked an FAE, it is reasonable to imagine that, like the biobots, they could exist and flourish as autonomous organisms. If this were the case, any embellishments brought about by later morphogenetic propensities or function-generating capabilities could only enhance the ability of successors to explore and create new affordances (Gibson, 1979; see below). The section that follows will describe how animals produce functionally specialized cell types.

THE METAZOAN-SPECIFIC FUNCTION-APPROPRIATION ENGINE

Animal embryos co-opt pre-existing gene co-expression networks (Singh *et al.*, 2018) into specialized cell types using a gene regulatory system different from those of all other forms of life. While the genes of all eukaryotic (nucleated) cells are organized into chromatin, a complex of DNA and histone and nonhistone proteins, the chromatin of holozoans has components not present in other eukaryotes, and the chromatin of metazoans has molecular features beyond those.

Transcriptional regulation in eukaryotic cells differs from the more familiar inducible and repressible modes of gene regulation of bacteria. Eukaryotes contain a “write-read-rewrite” transcription regulatory system (Prohaska *et al.*, 2010) in which the histone proteins that organize the cell’s DNA into chains of compact nucleosomes undergo reversible chemical modification, typically methylation and acetylation. Depending on the sites of modification, the altered histones have the capacity to restrict or facilitate access to associated protein-specifying or regulatory DNA sequences. This allows the genetic material to retain an epigenetic record of the “experiences” of the respective cells that can subsequently be edited or overwritten. Although not all eukaryotic cells exist in multicellular developing systems, those that do, e.g., in animal embryos, have their specialized fates determined by using this read-write system.

Transcriptionally active stretches of protein-encoding DNA are associated with Mediator, a large protein complex of 21–26 subunits which serves as a scaffold for expression by organizing relevant transcription factors and cofactors at the genes’ promoters (Harper & Taatjes, 2018). The complex’s subunit proteins have regions which are intrinsically disordered, causing Mediator’s interactions with the regulatory proteins it recruits (many of which have disordered domains themselves) to be affected by determinants additional to the proteins’ sequences. Like the epigenetic read-write system, the similarly pan-eukaryotic Mediator complex takes on novel roles in embryonic development in animals.

A major basis for this is the existence in metazoans and association with Mediator of two closely related proteins, p300 and CBP (derived from a homolog that first appeared in non-metazoan holozoans) which, by acetylating nucleosomal histones, amplify the activating branch of the read-write system. These proteins, in turn, recruit enhancers to liquid-like (because of the unstructured nature of many of the component proteins) hubs termed “topologically associating domains” (TADs: (Furlong & Levine, 2018) that behave as immiscible liquid droplets (reviewed in Newman (2020a). TADs typically contain one to three genes, and if only a single gene is present it is usually one that plays a significant role in development (Long *et al.*, 2022). This role might be the initiation of a cell lineage, for example, in which case the TAD will contain “pioneer” transcription factors which open chromatin regions for expression (Iwafuchi-Doi & Zaret, 2016), and lineage-determining transcription factors (LDTFs: (Obier & Bonifer, 2016).

For some early evolved, developmentally fundamental lineages, a single protein serves both pioneer and lineage-determining roles: Grainyhead for epithelium and Twist for connective tissue (Boivin & Schmidt-Ott, 2018), for example. In later diverging species, however, origination of new cell types came to depend on the synergistic effects of generically acting pioneer factors (e.g., Pax7) and LDTFs without pioneer function (e.g., Nkx-3.1 for heart muscle, Sox9 for cartilage), a more versatile combinatorial arrangement.

Independently of the composition and assembly of TADs, “tethering” DNA elements facilitate interaction of enhancers and promoters over long chromosomal distances, enabling the coordinated expression of multiple genes (Batut *et al.*, 2022). These “co-expression domains” (Soler-Oliva *et al.*, 2017) sometimes contain “super-enhancers” in which multiple enhancer elements mediate the elevated expression of genes associated with specialized cell types. The “cell type-specific core regulatory complexes” of Wagner *et al.* (2019) can thus be identified with tethered multi-TAD nuclear domains.

This mechanism of heightened and coordinated gene expression is lacking in Placozoa due to the non-use of enhancers (see above) but it is present in all other metazoan phyla. Evolution can evidently lead to more elaborate versions. A comparative study of the embryos of an acorn worm (a hemichordate), amphioxus (an invertebrate chordate), and zebrafish and *Xenopus* (two vertebrates) showed the vertebrates to have a substantial increase (relative to the invertebrates) in the number of and functional connectivity of genes that change in expression level in response to externally administered developmental effectors. This was partly due to increased numbers of developmentally specific gene regulatory sequences, but it was also accompanied by significant differences in open chromatin domains with increased density of enhancers (Gil-Galvez *et al.*, 2022). This suggests that the increased phenotypic complexity, including functional complexity, of vertebrates relative to other animals (the inverse of the placozoan condition), might be related to a more sophisticated capacity to co-regulate genes. Each transition in inherent capability would represent an irreversible step in evolution, but also open possibilities of further elaboration within a phylogenetic lineage.

Because essentially all the functions recruited and amplified by this multi-TAD, multi-enhancer system can be traced to intrinsic functionalities of ancestral holozoan cells, and few, if any, cannot be, it can be considered (as noted above) a metazoan function-appropriation engine. Much evidence suggests that the FAE amplifies life-sustaining activities that were already present in the unicellular and transiently colonial holozoan cell populations they arose from, and partitions them into specialized cell types. How the partitioning is accomplished is poorly understood, but in one case, a cytoskeletal protein appears to act as a switch between two lineage-related cell types – skeletal muscle and adipocytes (Zhao *et al.*, 2013). Further, which cells in an embryo acquire specific functions and how these cell types are arranged is not determined by the FAE itself, but by an array of morphogenetic and pattern-forming mechanisms based on self-organizational and other material inherencies of developing tissues (see the introductory section, and Newman (2019).

Cell types with evolutionary roots in the functionality-associated co-expression networks of unicellular ancestors include epithelial cells (protection), myoblasts (motility), connective tissue fibroblasts, osteocytes, and chondrocytes (extracellular matrix production), neurons (electrical excitability), hepatocytes (detoxification), adipocytes (lipid storage), retinal rods (light responsivity), and erythrocytes (oxygen capture) (Table 1). If metazoan tissue and organ functions have been recruited, as suggested, from functionalities of cellular ancestors, those found only in distant lineages would not be present in animals except for rare cases of endosymbiosis or lateral transfer of genes. This conjecture is consistent with the absence in animals of cell types that capture light for photosynthesis or store starch, although they are abundant in the vascular plants. The cellulosic tunic of urochordates appears to represent an exceptional laterally transferred skeletogenic functionality (Nakashima *et al.*, 2004).

[Table 1 about here]

CONCLUSION: FUNCTIONS AS INHERENT, ENTRENCHED OR OPTIONAL

This paper began with a discussion of the concept of function in biological theory and its importance for understanding organic evolution. It was seen how during the 20th century the mainstream of philosophical thinking on the subject moved from a focus on natural law modelled on explanation in the physical sciences, to one emphasizing organismal capabilities and their purposes. A purposeful function was conceived not teleologically (that is, tending toward an externally imposed end) but in two other senses: as the causal role that an embodied process plays in maintaining the characteristic life of an organism, and as a process fashioned during the history of the organism’s lineage which has led to its being the kind of organism it is. (Both of these senses have been referred to as “teleonomic” by various writers; see Corning (2019).) As argued by (Buller, 1998), the second, etiological sense of function presupposes its having a causal role in its present-day form.

Currently, the etiological notion of function is the prevailing one in the philosophy of biology. Many theorists have followed Millikan (1989) to claim that only those processes that have been acquired evolutionarily are “proper functions,” with all other embodied organismal activities presumably left to be accounted for by the older natural law type of explanation. This raises the question of how a proper function evolves to become incorporated into an organism’s identity. The default (or only considered) explanation is natural selection, the mechanism posited by the Darwinian-populational biological theory that holds that complex traits arise by successive accumulation of small variations over many generations due to differences in fitness (i.e., propensity to contribute higher numbers of offspring to the next generation). For this reason, (proper) functions have been conflated with “selected effects” (Neander, 1991).

This paper has contested much of this discourse about function, drawing on the concepts of inherency, previously advanced in relation to the evolution of form, and agency, a set of self-directed capabilities of all living cells that is manifested in different ways in derived multicellular entities. The idea that biological forms and their constituent morphological motifs are expressions of inherent dispositions of living materials (Newman & Bhat, 2009; Newman & Comper, 1990) has informed proposals that categories of organisms can constitute natural kinds (Newman, 2020b), and their individual exemplars, Kantian natural purposes (Moss & Newman, 2015).

When function is considered in terms of inherency and agency the concepts of teleology and teleonomy are similarly placed in a new light. Peter Corning has written about the rejection of the traditional (Aristotelian or religious) notion of externally imposed (teleological) goals by modern biology and the substitution of an organism-based (teleonomic) concept (Corning, 2019). Teleonomic goals are assumed to be products of evolution, but in some versions this attribution has been restricted to genetic mechanisms (“ultimate” causes) to the exclusion of organismal functions in all the senses described above (“proximate” causes), and in others to include the organism’s functional attributes and behaviours. Corning favours an expanded version of the latter model, a “means-ends” teleonomy in which agential organisms, in fashioning novel goals subvert the ultimate-proximate distinction. I suggest that the idea of inherency goes even further by reintroducing a form of teleology to the evolutionary process. First, as novel forms of matter, multicellular organisms such as animals, plants, and fungi occupy “possibility spaces” in which new forms and physics-dependent functions are preferentially accessed with small genetic or environmental variations. (The formation of the limited set of disparate, physically discrete chemical elements as the universe cooled is an analogous “preordained” evolutionary process.) Further, the collection of physiological functions that have appeared during animal phylogenesis were also based on inherencies, in this case in the life-sustaining activities of ancestral holozoan cells. The evolutionary unfolding of specialized cell types and organs was thus more a kind of teleology than teleonomy.

The view that functions are based on inherent properties of multicellular entities draws on the following inferences from studies reviewed above:

1. Multicellular organisms (animals are discussed here, but also plants, fungi, and aggregative microorganisms) are distinct kinds of matter. These materials have specific modes of self-organization which function to maintain their coherence, to define their potential states, and to bring about transitions between these states. When new genes or pathways are added to descendent lineages, new material properties can appear. Some of these mediate development and others characterize established tissues. In such complex materials no useful distinction can be drawn between material and efficient causes, causal role functions, and ancestrally survival-promoting (i.e., “proper”) functions.
2. Multicellular organisms (e.g., placozoans) lacking the requisite morphogenetic and gene expression toolkits for generating highly specialized cell types (e.g., muscle or nerves) and complex organs can instead use the dynamical properties of their tissues to accomplish essential behaviours such as locomotion or reproduction. Since these physical properties are all-or-none, there is no plausible scenario for their gradual emergence.
3. The cells constituting multicellular organisms and those they evolved from (non-metazoan holozoans for the animals, but also amoebae, bacteria, etc.) exhibit agential behaviours. Cells from dissociated embryos or organ primordia, or tumours, can perform behaviours unlike any in the evolutionary history of their source tissues. This includes probing channels in mazes in advance of entering them to make accurate choices between productive pathways and dead ends (Tweedy *et al.*, 2020). Re-aggregated cells, moreover, can exhibit agential behaviours still different from the cells that compose them, despite having no morphological or functional embellishments. Speculatively, if they could acquire new structures or specialized cell types by fiat, it is reasonable to expect that their behavioural repertoires would be enhanced. As Stuart Kauffman has long emphasized (Kauffman, 2019), biological novelties have no pre-set functions: “[t]he possible uses of objects for various purposes simply cannot be anticipated” (Felin & Kauffman, 2019).
4. Focusing on evolution as it actually took place, what, other than cycles of adaptive natural selection, could have equipped early appearing metazoan organisms with novel features? Regarding form, we have proposed it was the exploration by developmental processes of previously unaccessed, or newly available (owing to mobilization of new physical effects by novel genes) regions of the respective morphospace (Bhat *et al.*, 2016; Newman, 2016). For functions, I have suggested here it was, at the start, the emergent dynamical properties of metazoan cell masses: the surface tension-driven rounding-up of cell clusters leading to the canonical starting point of development of most species; in placozoans, new motile and reproductive functions based on the mechanochemical excitability of unbound metazoan cell sheets; and in eumetazoans the liquid crystal-like intercalation of cells and consequent elongation of embryos and appendages (reviewed in Forgacs and Newman (2005). Finally, novel functions arose by the chromatin-based cell functionality appropriation engine was poised to generate new cell types in all animal groups apart from Placozoa.
5. Organisms bearing novel morphological or functional features resulting from any of the described processes will survive, or not, depending on the uses of the new traits. There is no basis for the notion that increased fitness (i.e., capacity to leave more progeny than cohorts lacking the new features) will be decisive in propagating them. In agential (i.e., all) organisms phenotypic novelties can serve as enablements for constructing new niches or detecting new “affordances.” The latter concept, first introduced by the perceptual psychologist James J. Gibson (Gibson, 1979), has been characterized by Denis Walsh, who has made agency central to his evolutionary philosophy, as “a joint property of a purposive system and the conditions with which it interacts…opportunities for, or impediments to, pursuit of a system’s goals” (Walsh, 2015: 163). [See (Vane-Wright, 2014) for a historical account of such approaches to evolution in an introduction to a collection of relevant contemporary treatments.]

Bringing inherency to bear on form and function allows the origin and evolution of the respective features to be considered outside a selectionist/adaptationist framework. In doing so, however, most of the enigmatic aspects of function are collapsed to the level of the individual cell. In the case of form, physical processes (some with counterparts in non-living materials and thus having predictable outcomes) can account for major morphological features (layers, segments, appendages) (Newman & Comper, 1990; Newman & Müller, 2005). But the properties that make tissues susceptible to these physical effects are strictly dependent on the incompletely understood living activities (motility, adhesivity) of their cellular subunits. In the case of function, the subcellular inherencies that are the bases of new cell types, and the chromatin mechanisms of their recruitment are also poorly characterized. Agency, an indisputably inherent property of individual cells which is even more obscure than motility or differentiation, is also exported to and transformed into novel modes of behaviour and cognition at the multicellular level (Arnellos & Moreno, 2015).

The cell-centred view advanced here leads to a ready division of functions as either essential or optional (or dispensable). Essential functions include those that are necessary to keep the organism’s cells alive. In those cells, they are either coincident with, or contain as a subset, those functionalities that serve as recruitable protofunctions of specialized cell types. They are essential because without living cells there is no multicellular organism. There are also organism-level essential functions. These are ones that appeared during evolution and are generatively entrenched (Wimsatt, 2015), such that the organism in question could not develop without them. Entrenched functions can be transiently implemented, like gastrulation or neurulation in vertebrates, or enduring throughout the life of the individual, like blood circulation or respiration. Optional functions, in contrast, are ones that the organism can live without, like gait, sight, or speech. They might be necessary for survival in some circumstances, but they are not necessary for life. They can be thought of as “add-ons” to an organism’s biological identity but could become entrenched as a lineage evolves. Thermogenesis, mentioned above, is a possible example of this. The taxonomic group Sauropsidae contains forms that are both exothermic (reptiles) and endothermic (birds). When the group diversified, warm-bloodedness was likely a physiological add-on in transitional forms that enabled occupation of previously unavailable niches (Newman *et al.*, 2013). The biology of present-day birds would undoubtedly not tolerate the loss of this capacity.

Contrasting placozoans with artificially constructed cellular spheroids also highlights the potential of optionality of function in evolution. The ability of the spheroids to nourish themselves on dissolved molecules (in a suitable tissue culture environment) via single-cell-based membrane transport suggests that the external digestive system of the placozoan may serve (or served in transitional forms) as an optional function, enabling these organisms to feed on microfaunal prey in addition to potential absorptive nutrition. In eumetazoan forms a similar, but internalized digestive system is functionally essential, since those organisms are too large and thick-skinned to be nourished by direct cellular means.

A precedent for the view that morphological and functional features of organisms are optional enablements or add-ons, rather than type-defining adaptations, can be found in a seminal paper by Erkki Haukioja, “Are individuals really subordinated to genes? A theory of living entities” (Haukioja, 1982). Haukioja assumes of organisms only that they must maintain themselves. He asserts that traits, including many genes, are tools by which organisms survive rather than being their defining characteristics, and that their value to the organism lies in their role in promoting sustained representation in a population rather than maximal relative representation, i.e., fitness. (See also the discussion of Haukioja’s separation of the “process of living” from the “process of change” in Vane-Wright (2014).)

Optional functions do not fit comfortably in adaptationist and fitness-based population genetics models. If any biological feature, e.g., a proper or selected-effect function, can only arise and take its place in the organism’s repertoire of traits by being stringently honed over many generations based on conferring reproductive advantage to its bearers, it is difficult to imagine that the organism can do without it. But in fact, they can do so, because of developmental plasticity but also their agency (Roth, 2018; Slijper, 1942). Like inherency, agency is a neglected factor in adaptationist evolutionary theory.

In his famous essay “The Organism as Subject and Object of Evolution” (reprinted in *The Dialectical Biologist*: (Levins & Lewontin, 1985), Richard Lewontin vividly made the case for incorporating agency into evolutionary biology:

The roles of the external and the internal are not symmetrical in Darwinism. Pre-Darwinian variational theories placed the internal forces of development in the dominant position and understood history as a consequence of development…In Darwinian theory the reverse is true. Historical forces are dominant, and development does nothing but provide the raw material for the forces of natural selection. The external chooses which of many possible internal states shall survive. Thus the developmental pathways that we see are the consequence of history, not its cause…But such a view gives a false picture of organic evolution and cannot successfully cope with the problems posed by evolutionary biology, for it ignores two fundamental properties of living organisms that are in direct contradiction to a superficial Darwinism. First, it is not true that the development of an individual organism is an unfolding or unrolling of an internal program…Second, it is not true that the life and death and reproduction of an organism are a consequence of the way in which the living being is acted upon by an autonomous environment. Natural selection is not a consequence of how well the organism solves a set of fixed problems posed by the environment; on the contrary, the environment and the organism actively codetermine each other. The internal and the external factors, genes and environment, act upon each other through the medium of the organism…The incorporation of the organism as an active subject in its own ontogeny and in the construction of its own environment leads to a complex dialectical relationship of the elements in the triad of gene, environment, and organism [pp. 88–89; 105–106].

Lewontin’s “active subject” refers to the whole organism, which is a fundamental locus, along with the external environment with which it interacts, in niche construction theory. (Indeed, these passages are credited with initiating this subfield of evolutionary biology, although Lewontin himself credited Peter Kropotkin for the perspective, and similar views can be found in the writings of Alfred North Whitehead.) In the present paper, in seeking the origins of organismal function I have focused instead on features of cells themselves: the physics of their aggregated states, the molecular systems by which their functionalities are recruited in multicellular forms, and their agency. Rather than a triad of gene, environment and organism, the relevant relationships are between the microscale of the cell nucleus, the mesoscale of embryonic tissues, and the capacity of an agential organism to actively inhabit its world enabled by the novel physiological outcomes resulting from the synergy of the first two.

Using the example of the artificially generated spheroids, I have suggested that simply being made of living cells endows an entity with agency. This “ground state” of multicellular agency depends at a minimum on ciliary motion and membrane transport, but probably more. The properties of this experimental system permit the inference that additional functions can serve as non-obligatory embellishments. These can arise by altered development to generate organisms with new capabilities for exploring new facets of the environment. Functions, understood in this way, need not be “proper,” selected effect, or, except in a few fundamental cases, have necessary causal roles in maintaining organismal integrity.

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DATA AVAILABILITY

This conceptual review is based on data available in the published literature.

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**Table 1 Ancestral cell functionalities as prototypes for differentiated cells**

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| --- | --- | --- |
| **Cytoskeletal- mediated motility** | Myoblast | Falk DL *et al*. 2003. Shared, unique and redundant functions of three members of the class I myosins (MyoA, MyoB and MyoF) in motility and chemotaxis in Dictyostelium. *J Cell Sci* 116: 3985-3999. |
| **Electrical excitability** | Neuron: axon | Cai X. 2012. Ancient origin of four-domain voltage-gated Na+ channels predates the divergence of animals and fungi. *J Membr Biol* 245: 117-123. |
| **Neurosecretion** | Neuron: synapse | Göhde R *et al*. 2021. Choanoflagellates and the ancestry of neurosecretory vesicles. *Philos Trans R Soc Lond B Biol Sci* 376: 20190759. |
| **Detoxification** | Hepatocyte | Martins M *et al*. 2010. Insights into the phylogeny of arylamine N-acetyltransferases in fungi. *J Mol Evol* 71: 141-152. |
| **Chitinous matrix** | Cuticle epidermal cells | Torruella G *et al*. 2015. Phylogenomics reveals convergent evolution of lifestyles in close relatives of animals and fungi. *Curr Biol* 25: 2404-2410. |
| **Light-responsive signal**  **transduction** | Photoreceptors of retina | Yoshida K *et al*. 2017. A unique choanoflagellate enzyme rhodopsin exhibits light-dependent cyclic nucleotide phosphodiesterase activity. *J Biol Chem* 292: 7531-7541. |
| **Na⁺/K⁺-ATPase osmotic regulation** | Kidney | Saez AG *et al*. 2009. Evolutionary history of Na,K-ATPases and their osmoregulatory role. *Genetica* 136: 479-490. |
| **Extracellular digestion** | Pancreatic and salivary  gland exocrine cells | Tikhonenkov DV *et al*. 2020. Insights into the origin of metazoan multicellularity from predatory unicellular relatives of animals. *BMC Biol* 18: 39. |
| **Lipid droplets** | Adipocytes | Renne MF *et al*. Lipid droplet biogenesis: A mystery "unmixing"? *Semin Cell Dev Biol* 108: 14-23. |
| **Haemoglobin** | Erythrocytes | Webster DA. 1988. Structure and function of bacterial hemoglobin and related proteins. *Adv Inorg Biochem* 7: 245-265. |