# Symbiogeny and the Evolution of Tissues: The Hypothesis

Stanley Shostak*

*Associate Professor Emeritus, University of Pittsburgh, Biological Sciences 2307 Pittock Street, Pittsburgh, Pennsylvania 15217*

*Corresponding author: Stanley Shostak , Associate Professor Emeritus, University of Pittsburgh, Biological Sciences 2307 Pittock Street, Pittsburgh, Pennsylvania 15217, United States, Tel: 0114129156595; E-mail:sshostak@pitt.edu

## Abstract

The symbiogeny hypothesis (1) attributes the origin of stem-metazoans to the formation of symbiogens of eukaryotic epithelial-like spheres fused with ameba-like cells and (2) credits the intra-organismic evolution of metazoan tissues and differentiated cells to competition, interactions, and selection among hereditable variations originating in symbiogens. Data consistent with the symbiogeny hypothesis are drawn from the fossil record of Doushantuo phosphites, molecular phylogenetics, tissue segregation and flexibility, and from the epithelial to mesenchymal and mesenchymal to epithelial transformations during development and malignancy.

**Keywords:** Symbiogeny; Symbiosis; Doushantuo phosphites; Epithelial Mesenchymal Transition; Mesenchymal Epithelial Transition

## Introduction

Metazoan organisms were the venue for the evolution of tissues, and differentiated cells were the fruits of cellular survival and reproduction within metazoans. The symbiogeny hypothesis borrows concepts of competition and selection from evolution by natural selection to explain the evolution of tissues and differentiated cells developing and maintaining themselves in the "biomes" and "ecosystems" of metazoan organisms [1–2].

Of course, "[n]atural selection can operate on a trait at any given hierarchical level provided the trait is heritable and covaries with fitness at that level… [even if] determining the appropriate level is challenging especially during transitions where selection is expected to act simultaneously at multiple levels" [3]. Symbiogeny thus fills the gap between intra- and inter-organismic levels in the evolution of metazoans.

Conceptually, symbiogeny at the intra-organismic level is closely related to Lynn Margulis' concept of symbiogenesis or serial endosymbiosis theory (aka symbionticism) at the intra-cellular level [4-7], i.e., the "process of the 'assembly' of a complex system from largely 'prefabricated parts'" [8]. Phylogenetic analyses "suggest that primary plastid endosymbiosis occurred ~900 Mya [million years ago] and mitochondrial endosymbiosis occurred ~1,200 Mya" [9]. Thus, symbiogeny could have begun somewhere in the vicinity of a billion years ago, since metazoans are eukaryotes equipped with mitochondria.

Symbiogeny proposes that fortuitously paired cryptozoic eukaryotic **symbiotes** entered into fused partnerships as **symbiogens** and together evolved into the variety of complex **symbionts** presently constituting the Metazoa (despite "very low bootstrap support" [10]). Nuclear fusion (one nucleus "swallowed" by another [11]) or horizontal (lateral) gene transfer from one symbiogen's nucleus (the nucleomorph) to the other (the dominant nucleus) would have permanently sealed the original partnerships.

Specifically, symbiogeny proposes that cryptozoic fusion(s) took place between eukaryotic epithelial-like spheres and individual ameba-like cells (or plasmodia) thereby creating proto-stem-metazoans (urorganisms or urmetazoans [12]). Essentially, the fusions brought "hunters" (ameba-like cells) into an epithelial "blind." The epithelial component of symbionts provided a reclusive internal environment through the action of apical terminal bars or junctional complexes (tight junctions and occluding zones) between epithelial cells in addition to their basal lamina.

For their part, in the protected environment provided by epithelia, the ameba-like cells experimented with their "hunter's" genetic apparatus and elaborated a rich array of derivatives: mesenchymal tissues and mesenchymally-derived cells (various connective tissues, vascular tissue, and blood and lymphatic tissues). In addition, the "hunters" shaped the "blind." Conspicuously, interactions between mesenchyme and epithelia shaped the formation of internal (digestive) and superficial organs (scales, feathers, hair), and induced complex neuronal structures from originally external epithelial cells (i.e., cells retaining the limiting membrane [epineurium] and cellular attachments [synaptic junctions]).

Ultimately, tissues with mixed epithelial/ameba-like cell heritage evolved. Intercellular attachment sites among osteocytes in osteons, for example, are epithelial qualities in otherwise ameba-like connective tissue cells. Likewise, the fusion of myoblasts (i.e., pre-muscle transitionally amplifying cells [TACs]) into sarcofibers is an ameba-like behavior, while mammalian skeletal muscle's peripheral membranes [epimycia], intercellular [sarcomeric] attachment sites, and satellite cells (adult stem cells [ASCs]) are epithelial-like.

In effect, "pre-adapted extant entities [merging] into a new whole… [possessed] a fundamentally different source of variation from the gradual accumulation of small random variations" [13] underlying Darwinian evolution. Consequently, the new configurations possessed sufficient heritable variety to bring about intra-organismic competition and selection. The hypothesis of symbiogeny, thus, offers "a new approach to…expanding evolution to an adequate level of integration" [14].

## Data Consistent with Symbiogeny

Working out the evolution of early metazoans through the fossil record and molecular phylogeny has been problematic. Working around the abundance of contradictory interpretations of data, however, leaves a clear path toward symbiogeny.

### The Fossil Record

Efforts to track traces of metazoan evolution in stone are steeped in controversy on the roles of taphonomy (changes in the trace of organism after death and during fossilization) and diagenesis (changes in the fossilizing sediment after deposition). Nevertheless, "[l]ate Pelaeoproterozoic and Early Mesoproterozoic rocks preserve evidence… of preservable eukaryotic organisms…[with the] capacity for generating morphological diversity" [15].

A great deal remains to be unearthed about the evolution of stem-metazoans, but symbiosis seems to have left its mark on the circular and frond-shaped fossils that emerged in the Ediacaran period. Indeed, the shallow water varieties in Australia, the White Sea, and Namibia of flat, "iconic, serially quilted erniettomorphs and fractally branched rangeomorphs" [16] might well have obtained nutrients from photosynthetic endosymbionts. The "sea-floor 'pancake'… may have lived with an internal 'garden' of symbiotic, photosynthetic, food-producing monerans or protists" [17].

That is not to say that symbiosis was universal. Indeed, the "snapshots" of stages in the succession of the deep-marine Ediacaran communities at Mistaken Point, Newfoundland (~565 Mya) lived unequivocally below the photic zone and could not have been photoautotrophic [18]. But symbiosis elsewhere would have been compatible with endosymbiosis and symbiogeny.

Other fossils bear suggestive, if not telltale signs of symbiogeny. Among the "superbly preserved heterogeneous assemblage of bacteria, cyanobacteria, planktonic algae, submillimetre-sized burrows and problematica" [19] of the lower Doushantuo phosphites from China's Yangtze Gorges especially from Weng'an, Guizhou Province China (~580 Mya) are odd couples of seemingly unrelated living forms bound together in spheroidal fossils. These spheroids, ~500 μm (100 to 700 μm) in diameter and frequently covered with an ornamented cuticle 10 μm thick contain an internal mass that appears to be either a single cell or groups of cells in low numbers displaying a $2^n$ or palintomic cleavage pattern (i.e., palintomy: successive reductive, binary division with little intervening growth).

The fossils exhibiting this pattern were initially interpreted as spheroidal volvocacean green algae. The algal interpretation was questioned, however, since the closely packed cells conformed to each other within their ornamented vesicle. Similarly, some of the Doushantuo Neoproterozoic phosphorites were interpreted as the remains of giant sulfur-oxidizing bacteria "consistent with the morphology, taphonomic robustness and paleogeochemical conditions required to explain… the preservation of other Doushantuo fossils" [20], but this interpretation was criticized because of the apparent presence of nuclei absent in bacteria, ornamented cysts, and the apparent multicellular contents of some fossils [21].

The fossils were also interpreted as encysting protists represented today by parasitic unicellular mesomycetozoea [22]. However, among other objections, the fossils' "ornamented envelopes… are entirely different from sporangium walls of modern mesomycetozoeans" [23].

The obvious interpretation of the spheroids, namely, that they were the eggs and embryos of metazoans had its supporters [21, 23-29] and detractors [22, 30–33]. On one side: "By far the closest morphological matches to these fossils are diapause egg cysts, or hulls, produced by a phylogenetically disparate array of aquatic invertebrates… [seen in n]ew collections from Xiaofenghe section of the Doushantuo formation… [showing] that cleavage, at least through the 16–cell stage, occurred within… vesicles… identical to other populations interpreted as early cleavage embryos" [29].

The solid spheroids were dubbed Megasphaera and regarded as animal eggs; spheroids partitioned one to four times became known as Parapandorina and were considered early cleavage stage embryos; the more highly cellular fossils, called Megaclonophycus, were alleged to be more advanced embryos [20, 23]. In fact, some of the "early embryos" were said to have a polar lobe much like contemporary spiralian embryos [21]. Remarkably, despite their spheroidal appearance, the fossils were interpreted as evidence of cladogenesis within the Bilateria before the appearance of diverse Cambrian assemblages.

The "egg/embryo" interpretation of the fossils was attacked for inadequate geophysical analysis, for a failure to adequately consider diagenetic artifacts, and for ignoring the morphological evidence that so-called embryos "composed of hundreds to more than ~ 1000 cells… show[ed] no evidence of blastocoel formation or the organization of blastomeres into epithelia" [27]. The fossils were, therefore, reinterpreted as nonmetazoan holozoans or possibly even more distant branches of the eukaryotic tree — unicellular eukaryotes evolving from the last common ancestor of animals and fungi. Rather than indicating embryonic development, the palintomic pattern of cell division was interpreted as producing a flood of asexual propagules (endospores). Indeed, "[w]herever the Doushantuo fossils eventually end up, it will clearly not be within 'crown-group' Metazoa" [31].

Symbiogenic conjectures might profitably enter the debate at this juncture: Might the Doushantuo phosphorites represent symbiotic combinations of proto-stem-metazoans in which epithelial-like spheroids contained ameba-like cells? Possibly "[t]he combined developmental evidence from the fossils indicates… mother cells [as symbiont or parasite], enlarged by hypertrophic growth… encysted with a multilayered envelope… [that] directly, or after a resting period began a process or coordinated mitotic palintomic cleavage (Paradandorina–Megaclonophycus). Eventually, germination occurred, during which the outer envelope wall ruptured, and the more pliable inner wall with its content emerged in finger-like protrusions, typically forming peanut-like objects. The cells resulting from the cleavage process escaped as propagules, probably through dissolution of the inner wall" [22].

Conceivably, the internal cells represent a "modern analog (a parasitic dinoflagellate) showing hypertrophic growth with constant karyoplasmic ratio followed by palintomic cleavage resulting in spores an order of magnitude smaller than the nuclei of the late hypertrophic stages" [33]. "[E]arly animal evolution may have involved a synzoospore stage without embryogenesis… [of unicellular eukaryotes] on the animal branch of the holozoan tree… [and] some Ediacaran macrofossils… [may have been] little more than upper and lower epithelia that lined a fluid-filled or mesogloea-like interior" [23].

Further analogies with symbiogeny can be drawn from thin sections of some "previously overlooked" phosphorite Megaclonophycus-stage specimens from the Doushantuo Formation at Weng-an containing,

"in addition to the blastomere-like cells… one or more spheroidal to ellipsoidal multicellular structures, here termed matryoshkas in reference to their similarity to nested Russian dolls. The matryoshkas are of variable size (30–350 μm) but they are generally larger than blastonere-like cells. They themselves are multicellular, consisting of tightly packed cells (9–14 μm in size) that are significantly smaller than the blastomere-like cells. Measurements show that the matryoshkas do not follow a palintonic cell division pattern… [Rather,] matryoshkas are growing structures, with cytoplasmic growth after each division to restore cell size" [34].

Ironically, these observations and conjectures make "strange bedfellows" of advocates and detractors of the "egg/embryo" interpretation and bring them into quasi agreement around symbiogeny: "At least some of the microfossils attributed to embryo-like populations… [may represent] distinctive reproductive propagules" [23]; "the peripheral cells are detached and form isolated structures that are consistent with a function as propagules" [33]. Indeed, "[t]he propagules are released through perforation or rupture of the wall of the cyst… or through germination tubes" [22]. In either case, the enclosed structures are consistent with symbiotes (or parasites) within a host epithelium.

## The Molecular Record

Molecular fossils in organisms (as opposed to petrified fossils in stone) are found in expressed sequence tags (ESTs), nuclear and mitochondrial gene alignments, sequenced transcriptomes and genomes. Base substitution, mutation, rearrangement, inversion, etc. are aligned in orderly scenarios and interpreted as evidence of linear evolutionary change while ignoring molecular taphonomy and diagenesis. When not totally embedded in circular reasoning, these same data can also reveal the roles of symbiogenesis in the evolution of eumetazoans.

### Epithelial-like ancestors

What sort of epithelial-like organisms would have been an originary symbiote in the evolution of metazoans? The problem answering this question arises from the difficulty tracing a purely epithelial-like ancestor back from existing organisms. What came first?

With the exception of Mesozoa, (e.g., endoparsites of Plathelminthes), throughout the Epitheliozoa [43], epithelia have specialized intercellular attachment sites (e.g., zonulae adherentes, septate desmosomes, macula adherens) and generally rest on a basal lamella. According to the "[c]onsensus view of phylogenetic relationships of the major metazoan lineages based on recent phylogenomic studies" [44] the Epitheliozoa consist of two clades of radiates, Placozoa/Ctenophora and Cnidaria, and the informally monophyletic Bilateria.

As a first approximation, the "ancestral" type at the root of the epitheliozoan tree would not have resembled modern Cnidaria, since "[r]ecent studies and a critical revaluation of old knowledge disclosed the growing view that the origin of triploblasty predated the cnidarian-bilaterian divergence" [45]. What is more, "several important classes and subclasses of homeodomains… appear to be absent" when "a set of 76 homeobox-containing genes… [from a high-quality rough draft of the genome of the ctenophore *Mnemiopsis leidyi* and] phylogenetically categorized… into established gene families and classes and then compared to… the homeodomain repertoire of

species from the other four early branching metazoan lineages" [46]. Thus, stem-placozoan/ctenophors are deemed basal to cnidaria [47].

In addition, ctenophores' ribosomal RNA shows little resemblance to that in cnidarians or bilaterians thereby placing Ctenophora "in a class of its own." Conspicuously, like sponges, ctenophores lack a "set of synaptic scaffolding genes… all of which are present in cnidarians and bilaterians"[48]. And despite the presence of muscle in ctenophores, the ctenophore transcriptome contains "almost none of the genes involved in bilaterian mesoderm development" [48]. In fact, "[f]unctional components of the fibroblast growth factor, notch, hedgehog, and the nodal (TGF-β superfamily) pathways, all of which are important in the segregation of mesoderm in different bilaterian forms, are also *not observed* [in ctenophores]. Other genes known to be involved in bilaterian mesoderm development, such as gli/glis genes, are expressed in neural (but not mesodermal) cells" [48; emphasis added]. Thus, Ctenophora even falls out of the Radiata thereby demolishing the notion of radiates as a monophyletic sister clade to Bilateria [but see 49], and Ctenophora replaces Porifera as the sister clade to Bilateria [43-44, 50–52].

That leaves the placozoan *Trichoplax* as the "'living fossil' [and] relict of an early stage of animal evolution" [53]. Based on an "analysis of a concatenation of 104 slowly evolving single-copy nuclear genes (6,783 aligned amino acid positions) drawn from nine diverse fully sequenced genomes… placozoans are found to be a sister group to the other eumetazoans… with demosponge sequences diverging before the *Trichoplax*-cnidarian-bilaterian clade" [53]. Indeed, "[a]t least from a genomic perspective, *Trichoplax* retains many ancestral features of its last common ancestor with cnidarians and bilaterians, which lived in the Precambrian" [53].

Placing Placazoa at the bottom of the epitheliozoan lineup is also justified on the basis of "the sum of morphological evidence, the secondary structure of mitochondrial ribosomal genes, and molecular sequence data from mitochondrial and nuclear genes that amass over 9,400 phylogenetically informative characters from 24 to 73 taxa… [t]ogether with mitochondrial DNA genome structure and sequence analyses and Hox-like gene expression patterns… [Thus,] Placozoa are basal relative to all other diploblast phyla" [12].

To the degree that the modern *Trichoplax* is representative and informative, therefore (despite its "surprising diversity at the DNA level" [53]), *Trichoplax*-like epithelial spheres would have been present in stem epitheliozoans. Members of one stem family would have given rise to the epithelial placazoan (lacking crucial mesodermal components and neural elements), while other stem epitheliozoans would have entered symbiogenic relationships with ameba-like cells giving rise to the non-bilaterian (radial) and bilaterian eumetazoans.

### Unicellular ancestors

Regrettably, the preponderance of molecular literature "confirms" (irony intended) the linear descent of early metazoans from small, single cell eukaryotes. Indeed, metazoans are typically said to have sprung from Choanoflagellatea [10, 12, 35–36] or representatives of a proposed phylum, Choanozoa [15, 37–38]. The identity of the alleged ancestral eukaryotic cells remains unclear, however, and sufficient ambiguity surfaces in the literature to allow the possibility of metazoans arising from a symbiogenic combination of cells from more than one source.

As proposed, Choanozoa contains three classes:

1) Ichthyosporea (mesomycetozoan parasites [*Icthyophomus*] and saprotrophites [*Corallochytrium*]),

2) Filasterea (with branched, very slender long-non-tapering "tentacles" [the Ministeriidae with symmetric radiating tentacles {e.g., *Ministeria vibrans*} and the Capsasporidae, symbionts with lateral tentacles and a specialized feeding peduncle {e.g., *Capsaspora owczarzaki*, an endosimbiont of the pulmonate snail *Bomphilaria*}]),

3) Choanoflagellatea (represented by *Monosiga brevicollis* and *Monosiga ovata*).

The Filasterea, Choanoflagellatea, and Animalia (Porifera and Eumetazoa) are sometimes combined informally in the filozoa. The addition of the Ichthyosporea to the filozoa defines the Holozoa [37].

Originally, the choanoflagellates were awarded the place of honor as proxies for the unicellular source of multicellular animals. The choice was suggested by the morphological resemblance of choanoflagellates to the sponges' collar cells, but molecular dissection and reconstruction of intron-rich genes and other molecular evidence attest to the choanoflagellates living up to their reputation [39]. Other results eliminate the Ichthyosporea and Capsasporidae as linear ancestors of metazoans, since both these lineages seem to have diverged prior to the origin of the choanoflagellate/sponge common ancestor [10].

The encoded proteins of the choanoflagellate *Monosiga brevicollis* demonstrate that the species evolved on its own and is not degenerate eumetazoan. Rather, "genomic features shared by *M. bevicollis* and metazoans were probably present in their last common ancestor… [including genes] central to cell signalling and adhesion processes in metazoans, suggesting a role in the origin of multicellularity" [39]. In addition to their caged flagella, *M. brevicollis* has basement membrane laminin encoding domains, and extracellular matrix proteins, including "at least 17 integrin-α-domains… [and] five collagen-domain-encoding genes… in an arrangement similar to metazoan collagens" [39].

Indeed, the choanoflagellates would seem well "preadapted" ("preapted") or, in Stephen Jay Gould's language, "exapted," for "evolvability" by the presence of "features initially evolved for other reasons" and available for "future cooptation" [40]. However, *Monosiga brevicollis* lacks integrin-β-domains and offers no evidence of stable cell adhesion [37, 39].

In contrast, the single-celled filastereans *Ministeria vibrans* and *Capsaspora owczarzaki* have integrin-β-domains along with "many components involved in cell adhesion such as crumbs, cadherin, focal adhesion kinase" [37]. What is more, *Ministeria vibrans* can be successfully cultured in aggregates as well as in dispersed cell cultures [37], and its adhesive genes, along with those of Capsaspora in combination with those of choanoflagellates are suggestive of a "'metazoan-origin' domain set… comprise[ing] the key innovations relevant to the evolution of complex multicellular development" [41].

The "abundance of some of these [multicellular] domains [in filastereans]… [including] laminin-type epidermal growth factor-like, Integrin-ß4 domain [etc.]" [41] is often discussed in terms of gene duplication, domain fusion, domain shuffling [37, 39, 41], with transposon insertion, retroviral transduction, transformation, and, epigenetic effects lurking in the background as possible mediators of differential gene action. But none of these possibilities accounts for bringing foreign genes together in a common genome; symbiogeny does.

Miraculously, the accumulation of adhesive genes in a cell, conspicuously integrin-α-domains and integrin-β-domains, are said to have taken "their modern recognizable form in the last common ancestor of filozoa, after it separated from Ichthyosporea" [37]. Clearly, symbiogeny deserves mention here as a possible alternative mechanism for bringing the different genes together! Horizontal (or lateral) gene transfer among symbiogens is too obvious to be ignored! Indeed, the "abundance" of adhesive domains can be accounted for by symbiogeny as easily as the endosymbiotic transfers and integration of mitochondrial and chloroplast genes into the eukaryote nucleus [9].

Ironically, even calls for radically reforming gene theories of evolution fail to consider anything approaching symbiogeny as a mechanism of evolutionary change [42–44]. However nothing whatsoever in the literature on metazoan origins from eukaryotes [44–53] eliminates the possibility of symbiogeny playing a role, and one can easily imagine how symbiosis (parasitic infection or hybridization) could be behind combining all sorts of domains within cells.

## Developmental Consequences of Symbiogeny

Symbiogeny is not intended to reclaim Ernst Haeckel's much-maligned Biogenetic law: "Ontogenesis is a brief and rapid recapitulation of phylogenesis, determined by the physiological functions of heredity (generation) and adaptation (maintenance)" [54]. But symbiogeny would inevitably leave its mark on ontogeny, and one would expect to find traces of symbiogeny reflected in development and homeostasis.

Presumably, epithelia would undergo a more conservative evolution than ameba, since epithelial traits would be constrained by a hostile external environment and would tend to "keep a good thing going," whereas amoeboid traits would be free to experiment radically in the safe and guarded internal environment provided by epithelia. Indeed, the literature of developmental biology demonstrates both these trends along temporal and spatial axes.

### Segregation

Symbiogeny's consequences in diploblastic (didermic) Cnidaria are apparent in the segregation of separate epithelial and ameba lines. The spatial segregation of tissues is complete whether developing by sexual reproduction as a planula larva or by vegetative budding. Once established, "never the twain shall meet" [but see 55].

In hydras, the epidermal (ecodermal) and gastrodermal (endodermal) epithelia consist of polarized, adherent cells mounted on opposite sides of a basal lamella (mesoglea). Ameba cells (aka interstitial cells) are individual, small and densely basophilic with relatively large nuclei. These cells generally occupy interstices at the base of the epidermal cells (interstitial sites) in hydra, while in polyps and medusas of other cnidarians ameba cells occupy expanded inter-epithelial compartments as well.

As demonstrated by numerous experiments, hydra's epithelia are incapable of producing ameba cells [56-57]. Specifically, hydras partially or fully depleted of ameba cells in any of a number of ways (e.g., treatment with colchicine, nitrogen mustard, hydroxyurea, urethane, or lowered temperature) suffer numerous losses of specialized cells, and the animals do not move or capture prey but tend to enlarge and grow supernumerary tentacles. These so-called "epithelial animals" do not restore the missing ameba cells, but when ameba cells are allowed to reestablish themselves, for example, after

grafting normal tissue to an epithelial animal, the animal re-acquires the missing specific cell linages, including eggs [58] and sperm [59] as well as normal behavior.

Some tissues of bilaterians show preponderantly epithelial-like qualities, while other tissues show preponderantly ameba-like qualities. For example, the epidermis and the intestinal lining share epithelia qualities such as intercellular attachment sites and external lamina, while connective tissue, mesenchyme, and vascular tissues are ameba-like by way of secreting extracellular molecules, exhibiting phagocytosis, performing cell movement through a medium or across cellular barriers, and recruiting related cells to sites of cell differentiation.

Epidermis is restricted to forming hard and soft keratin, and intestinal lining cells differentiate as absorptive cells or along a narrow range of secretory cells, whereas mesenchyme is endlessly innovative. Blood and lymphatic cells, in particular, come in a variety of sizes, shapes, and functions while contributing to homeostasis, wound healing, regeneration, remodeling, and immune functions. Furthermore, the formation of multi-nucleated connective tissue cells (e.g., osteoclasts) and platelet-forming blood cells (megakaryocytes) is also reminiscent of ameba-like cell behavior.

The temporal separation of embryo/larva and adult stages is another feature of development reflecting symbiogeny. Indeed, morphological dissimilarities of embryo/larvae versus adults are the crux of Donald Williamson's concept of larval transfer by hybridization [60] and Eric Davidson's concept of "set-aside" cells [61]. Both concepts propose that unique, non-embryonic/larval cells are sequestered during embryo/larval development and form adults after receiving permissive developmental cues.

"[O]ften referred to as the 'imaginal rudiment'… [the cells] are set aside from participation in embryogenesis itself" [61]. Indeed, a "new morphogenetic world was then created, one that was freed of the developmental constraints of quantal cell lineage, immediate embryonic specification and intrinsic size limitations; and as history shows, one that is capable of great variety in the use of morphological space" [61].

In contemporary metazoans, the isolation of cells with "set-aside" properties may occur early and stereotypically, for example, in the Spiralia, or only slowly and cryptically in the form of embryonic stem cells (ESCs), for example, in mammals [62]. The set-aside cells may be micromeres (e.g., annelids), imaginal disk cells (e.g., arthropods), or various types of progressively restricted stem cells (e.g., mammals) testifying to parallel routes of evolution of tissues and differentiated cells in metazoans.

## Flexibility

The introduction of ameba-like cells into epithelial-like spheres coincided with the acquisition of additional developmental refinements: flexibility and interactions between cell types. Notably in the Bilateria, the evolution of amoeboid cells as mesoderm promoted flexibility even in epithelia (as witnessed the parenchyma of organs [chiefly digestive but including epidermal appendages]) and released the cornucopia of adult epithelial and mesenchymal derivatives of set-aside cells and ESCs. Conspicuously, flexibility and interactions between epithelial- and ameba-like cells are prominent features of the phenomenon known as induction. The induction of the central nervous system in vertebrates is testimony to the productivity of interactions made possible by symbiogeny.

Another feature of flexibility is the transitions between epithelial and ameba-like (i.e., mesenchymal) qualities of cells and vice versa. Some cells switch once between epithelial and mesenchymal properties or mesenchymal and epithelial properties, and some cells switch more than once. These switches are known as the epithelial-mesenchymal transition (EMT) or mesenchymal–epithelial transition (MET).

The sea urchin, *Lytechinus pictus*, for example, provides an example of an EMT at gastrulation when large, tightly bound polarized micromeres in the blastula's vegetal plate epithelium start "hopping." These cells ingress into the blastocoel as primary mesenchymal cells destined to contribute to the skeletogenic primary mesenchyme.

An EMT occurs much later in vertebrate gastrulation. For example, in chicks, an EMT occurs at the streak stage when flask-shaped surface cells deepithelialize and penetrate the ectoderm's basal lamina. The cells ingress and then undergo a MET, coalescing to form the hypoblast and reepithelializing to form endoderm. Later, epiblast cells undergoing an EMT move through the definitive primitive streak and node and form mesoderm of multi-layered mesenchyme in the intermediate zone between the ectoderm and endoderm.

Coupled METs and EMTs take place during vertebrate somitogenesis (formation of somites). The paraxial somitic mesenchyme undergoes a MET, condensing and epithelializing as cells broaden their intercellular contacts. Central epaxial cells then undergo an EMT, de-epithelializing and forming the sclerotome, while the remaining epithelial portion of the somite grows, folds dorso-medially and forms the myo/dermatome.

More complex EMT/METs take place when neural crest cells are extruded at the junction of neural and cutaneous ectoderm during neural tube formation. In the head, the deepithelialized neural crest cells compact to form neuronal ganglia, the dental papilla of teeth, osteoblasts in portions of the cranial skeleton, and neuro-secretory cells. In the trunk, the cells migrating toward somites accumulate in dorsal root ganglia; cells migrating between somites form the primary sympathetic ganglia and the orthosympathetic chains; cells moving farther ventrally become sensory and motor ganglia of the peripheral nervous system and adrenaline-secreting cells of the adrenal gland. Other neural crest cells migrating in the skin become the ectomesenchyme of dermal papilla of hair and feather follicles, melanocytes, sensory cells, and members of the diffuse neuroendocrine system [See 63 for details and further examples].

Thus, EMTs and METs are neither rare nor trivial events in normal development. They represent developmental cartwheels as cells move through alternative states. Moreover, pathological changes, such as carcinogenesis would seem to be instances of the cartwheel becoming stuck or spinning out of control. While an evolutionary history of competition and selection among normal tissues resulted in their survival and reproduction, the cartwheel's failure to settle down normally undermines the organism's chance for survival and reproduction.

## Cancer

Carcinogenesis is frequently described in the language of abnormal EMTs and METs. Tumor cells are spoken of as transformed normal cells, and tumors are microscopically graded and clinically staged for benignity and malignancy along a transitional continuum. Thus, the language of cancer etiology speaks of alterations — hypertrophy and hyperplasia, dedifferentiation and atypicality, metaplasia, neoplasia,

and, pleomorphism — and the language of pathology identifies clinically relevant transformation — polyps vs. carcinomas, fibroma vs. fibrosarcomas, lipoma vs. liposarcoma, etc.

Indeed, many tumors are mixed bags of cells — e.g., epithelial tubules and epithelial-derived mesenchymal cells in a pleomorphic adenoma — as if the cells are confused about their identity — while other tumors are said to have dedifferentiated to an embryonic state, and previously arrested embryonic-like cells are said to have somehow reemerged (e.g., in "blastomas" such as glioblastoma). In fact, the more bizarre the cancer cell (e.g., the size and shape of the nucleus), the closer the cell is to death.

In all these descriptions, the progress of cancers seems paradigmatic of cellular confusion. For example, confusion characterizes the changes in initially harmless "pre-cancerous" epithelia (e.g., carcinoma in situ) transformed into ameba-like cells that breach their limiting membrane becoming invasive, destructive, and metastatic in the process. Likewise, the dissemination of some carcinomas (e.g., breast cancer to peritoneal and/or ovarian sites) is attributable to sites recruiting circulating cells that would not otherwise windup there. Do these cells suffer from confusions in their epithelial-like and ameba-like qualities — their symbiogenic origins and evolutionary descent — manifest in the failure of their "cartwheel" to settle down in their normal cellular configuration?

Tumor progression, metastasis, invasiveness, and destructiveness locally and at ectopic sites testify to the ability of cancer cells to outcompete their normal counterparts in intra-organismic survival and reproduction. The competition between normal and tumor tissues is not necessarily straightforward, but symbiogeny suggests how tumor cells at the junction of transitions between epithelial and ameba-like cells might gain a competitive advantage leading to abnormal growth and development [64–65]. Were cancer cells vacillating between or stuck at an incorrect point of an EMT or MET, the cells' "dual identity" might expand their prowess and give them adaptive advantages in the their competition with their normal counterparts.

The notion of "dual identity" is long standing in the cancer literature. Carcinomas and vascular cancers (leukemias and lymphomas) have not totally erased their epithelial and vascular characteristics. The malignant cells retain molecular markers even after morphological markers have disappeared. Indeed, these cancers testify to their origins in otherwise normal cells. But while the underlying molecular biology of tumors is well studied — the action of oncogenes, mutations in tumor suppressor genes, silencing by small ribonucleic acids (smRNAs), and transcription modifications mediated by epigenetic factors in otherwise normal cells — the factors providing an ecological advantage for metastasis and tumor growth remain largely unknown. In theory, moderating or removing the cancer's dual identity might deny it an adaptive advantage and lead to salubrious results.

Thus, three points relevant to cancer emerge from the symbiogeny hypothesis: (1) A better understanding of cancers' roots in symbiogeny might suggest how to promote cancers' movement through an unsettled EMT or MET; (2) the notion of carcinomas, sarcomas, leukemias, and lymphomas as invading alien bodies misses the point that their competition with normal tissues is normal; (3) rather than waging a strategic "war" with our cancers and employing tactics that "kill, maim, and destroy," normal tissues' competitive edge might be enhanced, and their coming out on top in the competition with cancer would foster organismal survival and reproduction.

## Summary and Conclusions

Conceivably, symbiosis will soon become a "central principles of evolution" [66] and symbiogeny will emerge as the mechanisms that launched cells on their evolutionary journeys to tissues and differentiation in eumetazoans. Symbiogeny proposes that urmetazoans arose when formerly separate epithelia-like and ameba-like eukaryotes joined forces, first loosely as symbiotes and then fused as symbionts. Epithelia provided the isolation chamber known as the internal environment where the amoeboid guests evolved into mesenchym and thence, through competition and selection, into a variety of internal tissues. Externally, epithelia remained a stalwart barrier, while internally epithelia became a passageway for nutrients and the parenchyma of mesenchymally shaped organs.

Abundant data in both the fossil record and molecular phylogeny are consistent with symbiogenic speculations. Traces of symbiogeny are also found in the maintenance of epithelial and amoeboid traits, in inductive interactions, and in transitions between epithelial and mesenchymal qualities in the course of development. Likewise, cancers develop when cells with a confused identity acquire an adaptive edge over normal tissue and exploit compatible niches.

Thus, symbiogeny would seem to have interesting spinoffs for research on normal development and pathological change. The challenge confronting researchers is to interpret the effects of symbiogeny on the evolution of tissue and differentiated cells in order to understand normal growth and development and ameliorate their non-adaptive cellular behaviors at the organismic level.

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