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Multiscale Memory And Bioelectric Error Correction In The Cytoplasm-Cytoskeleton-Membrane System

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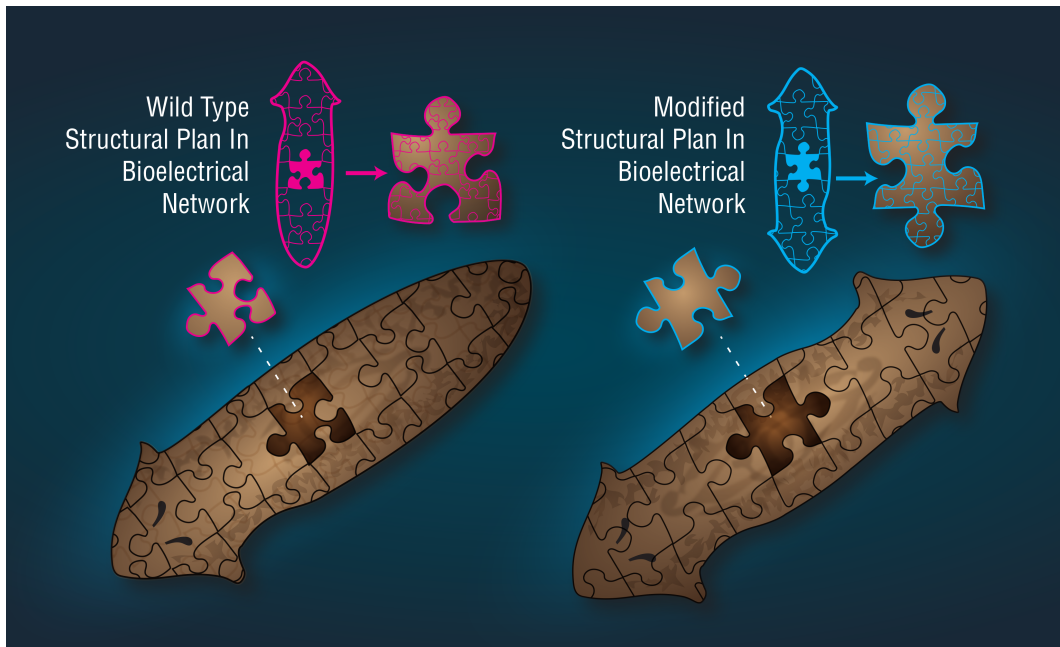
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Abstract

A fundamental aspect of life is the modification of anatomy, physiology, and behavior in the face of changing conditions. This is especially illustrated by the adaptive regulation of growth and form that underlies the ability of most organisms – from single cells to complex large metazoa – to develop, remodel, and regenerate to specific anatomical patterns. What is the relationship of the genome and other cellular components to the robust computations that underlie this remarkable pattern homeostasis? Here we examine the role of constraints defined at the cellular level, especially endogenous bioelectricity, in generating and propagating biological information. We review evidence that the genome is only one of several multi-generational biological memories. Focusing on the cell membrane and cytoplasm, which is physically continuous across all of life in evolutionary timeframes, we characterize the environment as an interstitial space through which messages are passed via bioelectric and biochemical codes. We argue that biological memory is a fundamental phenomenon that cannot be understood at any one scale, and suggest that functional studies of information propagated in non-genomic cellular structures will not only strongly impact evolutionary developmental biology, but will also have implications for regenerative medicine and synthetic bioengineering.

Graphical/Visual Abstract and Caption



Caption: The information determining large-scale anatomical features of an organism is encoded in a variety of physical processes and not entirely in the genome. Dissociations between genome-default anatomical states and actual growth and form can be observed in model systems such as planaria as pattern memories, including those stored in bioelectric networks among somatic cell groups, can be re-written to result in stable production of animals with different target morphologies after each regeneration event. Planaria, which reproduce by fission, starkly reveal the gap between genome and anatomy, as they maintain perfect anatomical fidelity even as they accumulate mutations over millions of years of somatic inheritance.

Introduction

What balances the various physical and biochemical forces that generate morphological patterns? In human constructions such as buildings or computers, forces are balanced by the architectural constraints that determine both spatial conformation and responses to perturbations, e.g. earthquakes or input data, respectively. Cells and multicellular organisms also have architectures that determine both spatial conformation and responses to perturbations (reviewed by (1)). By analogy with the genome, transcriptome, and proteome, we will use the term “architectome” to refer to the force-balancing architectural constraints that determine a cell's or organism's morphology and enable its dynamic behavior. The question of how the forces driving morphology are balanced becomes, in this case, the question of how this architectome is implemented. Relatively stable nucleic acid-protein complexes implement the genome as an informational structure, and relatively rapidly-varying RNA and protein concentrations implement the transcriptome and proteome as informational structures, respectively. The information encoded by these structures is not

sufficient to explain morphology, however. The genome does not directly encode three-dimensional shape, and transcription cannot distinguish large-scale spatial directions. For example, physical chirality (2-4) is necessary upstream of the asymmetric cascade of left- and right-specific factors in the ontogeny of laterality. Here we review evidence that the combined cytoplasm-cytoskeleton-membrane (CCM) system implements the architectome at the cellular level, and that cellular interactions implement it at the organismal level. Considerable evidence now suggests that evolutionary and developmental processes act at the level of the architectome, not just at the levels of the genome, transcriptome, and proteome.

Like the genome, transcriptome, and proteome, the architectome is a time-persistent informational structure, i.e. a *memory* (5) within any cell or organism. We trace the history of this memory structure from the last universal common ancestor (LUCA) to the present, showing that it encodes biological information over and above that encoded by the genome, transcriptome, and proteome (6). As Harold (1) and others have made clear, this result poses a strong challenge to the Modern Synthesis assumption that trans-generational biological memory is stored exclusively or even primarily in the genome (e.g., (7, 8)) and to its bioengineering corollary that a “bag of genes” or “bag of enzymes” is a reasonable architectural model of a cell (e.g., (9)). Morphological development is explicable within the conceptual scheme of the Modern Synthesis only if morphology mechanistically “emerges” from interactions between genome-encoded macromolecules. We review both experimental evidence and theoretical considerations indicating that this “emergence” picture is insufficient for developmental and synthetic biology and hence insufficient for bioengineering and biomedicine.

Paralleling the well-known role of electrical activity as the medium of computation in brains, developmental bioelectricity is increasingly recognized as a highly-conserved set of mechanisms for mediating the complex and robust pattern homeostasis of embryogenesis and regeneration (10-14). We suggest here that bioelectricity provides an error-correction mechanism for information encoded at the level of the architectome, and provide a perspective on the role of the bioelectric layer as a crucial component by which the genome regulates large-scale growth and form. Sexual reproduction forces the architectome to be compressed into a single zygotic cell. Given the role of endogenous bioelectric prepatterning in regulating spatial domains of gene expression and subsequent patterning, it can be hypothesized that ion channel locations on the cell membrane could provide a high-density encoding stabilized by bioelectric feedback. We predict below that ion channel arrangements are used ubiquitously to encode architectural information in zygotes. Disruptions of the CCM-encoded architectome alter cellular conformation and behavior; we suggest that some birth defects, cancers, and “connectome” disorders such as autism (15-17) can productively be viewed as architectome disorders. Understanding how to read, activate, and eventually construct architectural information encoded by the CCM system will enable new capabilities in regenerative medicine and synthetic biology (cf. (6, 18, 19)).

The CCM system implements an ancient, trans-generational memory for spatial architecture.

The CCM system is continuous from LUCA to the present.

While the process by which living cells originated remains controversial, there is broad agreement that all extant organisms are descendants of a single, unicellular LUCA. Deep phylogenetic trees or, if horizontal gene transfer (HGT) is explicitly included, networks are generally both intended and regarded as depictions of evolutionary lineages from LUCA; indeed such trees provide one of the primary conceptual tools for critically comparing alternative proposals regarding lineage (e.g., (20-22)). Trees or networks based on nucleic acid or protein sequences or other macromolecular characters implicitly reflect the modern-synthesis assumption that these molecules are the definitive indicators of lineage, i.e. that they are the sole evolutionarily-significant intergenerational biological memories.

Branch points in lineages represent cell divisions, even in the case of multicellular organisms (e.g. (23, 24)). Representations of the first two branch points of the universal, LUCA-rooted tree in which this is made explicit are shown in Fig. 1. These diagrams make explicit the assumption, which typically remains implicit (e.g. (21), Fig. 3), that LUCA was a *cell* with a membrane-enclosed cytoplasm. By depicting membrane and cytoplasm explicitly, Figs. 1a and 1b make it clear that the membrane and cytoplasm of LUCA are not only ancestral to but *continuous with* the membranes and cytoplasms of its bacterial and arkaryal (*sensu* (21)) descendants, as they must be if the branching between bacteria and arkarya is implemented by cell division. What differs between the models shown in Fig. 1a and 1b is the duration of the RNA world (alternatively, of a pre-“Darwinian threshold” (25) world dominated by HGT) and hence the biochemistry of the assumed cytoplasm. Similarly, Fig. 1c and 1d make it clear that bacterial membrane and cytoplasm are not just ancestral to but continuous with eukaryotic membrane and cytoplasm, as they must be on any plausible endosymbiotic model (26); the models shown differ in the extent to which bacterial membrane and cytoplasm are compartmentalized following the initial establishment of endosymbiosis. Hence all of life comprises, when viewed in time from LUCA to the present, one continuous cytoplasm contained within one continuous membrane.

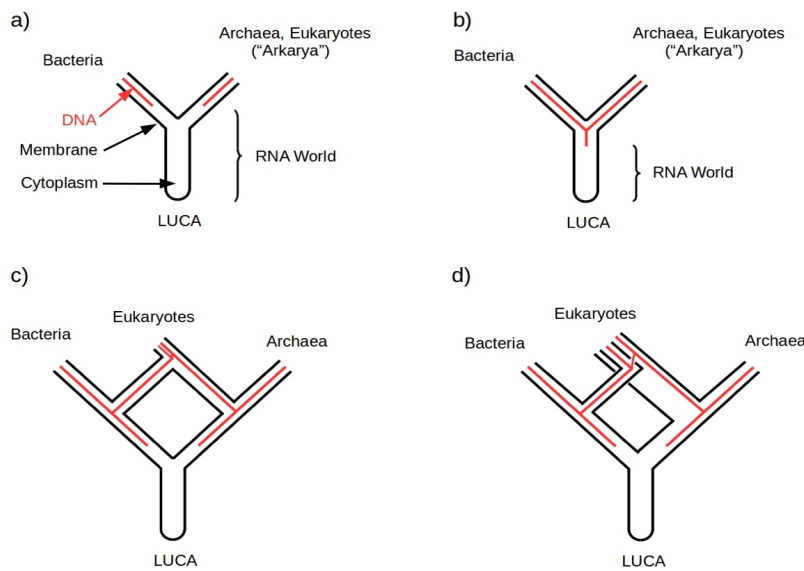


Fig. 1: Phylogenetic diagrams showing membrane (parallel black lines), cytoplasm (area between parallel lines) and DNA genome (red lines) explicitly; LUCA = Last Universal Common Ancestor. a) An extended RNA world hypothesis in which both Bacterial and "Arkarial" (*sensu* (21)) lineages independently evolve DNA genomes (cf. (21), Fig. 3). b) A shorter RNA world scenario in which both Bacterial and Arkarial lineages undergo massive DNA alteration and/or loss. In both cases, membrane and cytoplasm exhibit deeper temporal continuity than DNA. c) Endosymbiotic scenario for the origin of Eukaryotes in which Bacterial and Archaeal membranes and cytoplasm mix (cf. (26), Fig. 3). This diagram is consistent with the "ring of life" concept proposed by Rivera and Lake (176); see also (20). d) A more classical endosymbiotic scenario in which membranes and cytoplasm do not mix.

By depicting membrane and cytoplasm as continuous in time, the lineage diagrams shown in Fig. 1 emphasize the obvious point that these cellular components are never destroyed and then recreated from scratch (cf. (1, 27)). They can, therefore, encode information that is continuous through time. While bacterial and archaeal cytoskeletal components are much more diverse than those of eukaryotes (28, 29), they have similar structural and functional properties; hence it is useful to consider the cytoskeleton as a distinct component of the CCM system with its own ability to function as a biological memory. What information is encoded by this CCM memory? Intuitively, it is information about cellular *architecture*, what we have called the *architectome*. This information specifies 'what goes where' in order to enable the cell to function. It specifies the balance in the cellular balance of forces.

The information encoded by the CCM system is not generic.

The cell membrane is a physical boundary around the cytoplasm, and hence provides a *boundary condition* on the dynamic processes within the cytoplasm. Boundary conditions must be specified independently of the dynamics within the boundary in any system exhibiting feedback or other nonlinearities; that this applies to biological systems has been emphasized by Polanyi (30) and Rosen (31), among others. The significance of this boundary condition for evolution and development depends, however, on whether the encoded information contributes to evolutionary and developmental diversity, i.e. on whether it is *non-generic*. The CCM system as a whole similarly provides a boundary condition around the genome; whether this boundary condition is significant similarly depends on whether it is non-generic.

The information encoded by boundary conditions can be described using a precise mathematical formulation – the “Markov blanket” formalism introduced by Pearl (32) – and applied to biological systems by (33) (see also (34)). In any network of nodes with well-behaved causal interactions (formally, any network with ergodic dynamics), the Markov blanket around any node X comprises those nodes that directly influence X (“parents” of X), those nodes that X directly influences (“children” of X), and any other parents of X 's children as shown in Fig. 2a. The Markov blanket around X effectively “shields” X from the direct influence of the environment E outside of the blanket (formally, it renders X conditionally independent of E). The state of X at any given instant can be completely determined, in other words, given the state of the blanket at the immediately preceding instant. A Markov blanket can, alternatively, be thought of as mediating between X and E ; any causal interaction between X and E must flow “through” the blanket as shown in Fig. 2b.

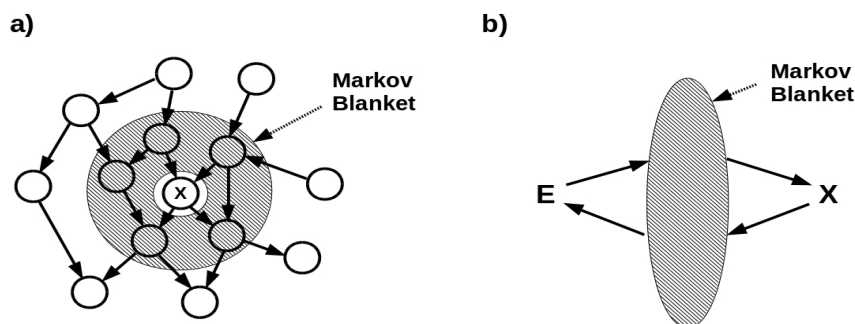


Fig. 2: Markov blankets provide a formal model of the CCM system. The Markov blanket separating a node X in a causal network from its external environment E comprises, by definition, the parents of X (nodes with arrows to X), the children of X (nodes with arrows from X) and any other parents of X 's children. a) example of a Markov blanket. Unlabeled open nodes comprise the external environment E of the “blanketed” node X ; nodes within the shaded area

comprise the Markov blanket of X. b) a Markov blanket can be thought of as “mediating” or “translating” between X and E.

The connections between the nodes in the Markov blanket around some given node X in a network determine how the node X interacts with the environment E outside the blanket. The blanket can, therefore, be thought of as encoding the information that specifies the X - E interaction and hence written as a pair of abstract mappings, $M: E \rightarrow X$ from states of E to states of X and $M^*: X \rightarrow E$ from states of X to states of E. If the “strengths” of the causal connections represented by the arrows are given by real numbers, the numbers of possible mappings M and M^* are infinite. Even if the causal connections are binary (on versus off), however, the number of possible mappings increases as 2^n where n is the number of arrows in the blanket. With just the seven arrows shown in Fig. 2a, there are 128 possible maps with binary connections; with 10 arrows, there are over a thousand, and with 20 arrows that number rises to more than one million. The probability that a Markov blanket encodes information specifying any given function thus decreases exponentially as the complexity of the blanket increases.

The CCM system of a cell can be considered to be a Markov blanket separating the genome from the external environment (33); technically the CCM system comprises multiple layers of Markov blankets). The number of arrows within this blanket is on the order of the number of biologically-significant biochemical interactions within the CCM, clearly a huge number. Diagrams of signal transduction pathways provide a partial representation of the Markov blanket between environment and genome; this familiar representation is only partial because *all* causal interactions occurring within the CCM contribute to the overall function of the blanket. The probability that the CCM system of a cell encodes any given mapping from the environment to the genome is, therefore, essentially zero. While the CCM systems of two very closely related cells, e.g. members of a clone, may encode very similar functions, the probability that the CCM systems of more distantly related cells, e.g. bacterial and archaeal cells, encode the same or even similar functions is again essentially zero. The information encoded by the CCM system is, therefore, *not generic*. Not only does the information content of the architectome increase with cellular and organismal complexity, but this information content is expected to be highly diverse even at a given level of complexity. Information encoded by the CCM system can, therefore, be both evolutionarily and developmentally significant; we show below that it is significant in fact.

CCM-encoded memories span 15+ orders of magnitude.

While the oldest memory encoded by the CCM system can be assumed to be the distinction between “inside” and “outside” that defined LUCA as a cell, the diversification of CCM systems across extant organisms shows that many CCM-

encoded memories are much more recent. Heterologous protein functional expression studies have shown that protein structure, which reflects such CCM-encoded properties as ion concentrations and water organization, has in some cases been conserved at least since the divergence between animals, plants and yeast (i.e., for over a billion years (e.g. (35))). The morphologies of and interactions between individual cells are in some cases transient, but in others may be preserved for decades, e.g. in mammalian nervous systems. Molecular concentrations and extracellular gradients may be stable from minutes to hours, and bioelectric phenomena may be stable from a few tens of milliseconds to hours. Hence CCM-encoded memories span roughly 18 orders of magnitude in time, as shown in Fig. 3.

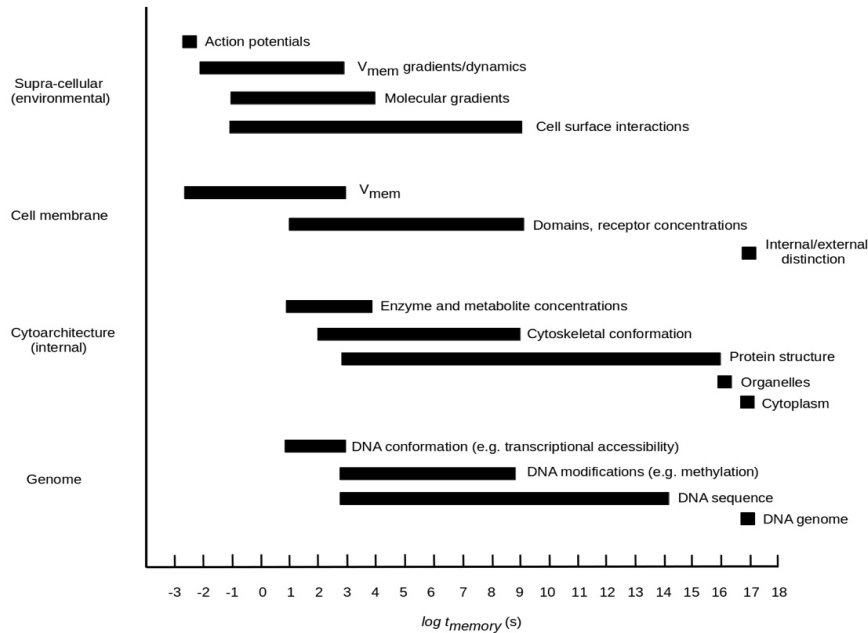


Fig. 3: Spatial and temporal scales of CCM-encoded memories. The smallest to largest spatial scales of CCM memories span roughly ten orders of magnitude; however, the spatial constraints on these memories extend to both smaller and larger scales.

The spatial scales of CCM-encoded memories exhibit a smaller but still significant range, from the nm dimensions of individual protein domains or the tens of nm dimensions of membrane raft structures to the meter-long processes of some mammalian neurons and tens of meters of large fungal colonies or trees. At every spatial scale, form depends on context defined at both smaller and larger scales (31). The small-scale constraints on morphology thus extend downward to the atomic scale and below, while the large-scale constraints extend to the

ecosystem and biosphere scale and above. The “fine-tuning” of these constraints in a way that makes organic life possible poses a substantial problem not just for origin-of-life studies but for high-energy physics and cosmology (e.g. (36)).

The architectome does not “emerge” from the genome.

As noted earlier, the Modern Synthesis assumes that morphology “emerges” from interactions between genome-encoded macromolecules. The concept of “emergence” is problematic, with many only partially compatible or even incompatible definitions and characterizations in the literature (e.g. (37-39)). In the context of the Modern Synthesis, the idea that morphology “emerges” requires that morphology not depend significantly on information encoded outside of the genome. Otherwise, the status of genes as the exclusive “replicators” that encode trans-generational biological information while supra-genomic structures are mere “vehicles” that transport genes from place to place (8) collapses. This idea of morphological emergence is often stated metaphorically by the claim that the genome is the “program” that directs cellular behavior (see (40) for an argument that this “metaphor” can be taken literally). Hutchinson *et al.* (41) state this assumption explicitly: “The genome sequence of a cell may be thought of as its operating system. It carries the code that specifies all of the genetic functions of the cell, which in turn determine the cellular chemistry, structure, replication, and other characteristics” (p. aad6253-1). It is crucial to consider the origin of the dynamics that allow single cells to orchestrate their activity toward creation and flexible repair of specific large-scale anatomical goal states (19, 42); where is the set-point of the remarkable pattern homeostasis encoded and how is it propagated across evolutionary timescales?

Is the biological information encoded by the genome, and by extension the transcriptome and proteome, sufficient to determine morphological outcomes? As Waris (43) put it regarding the desmid alga *Micrasterias*, “the important question (is) whether the cytoplasm possesses hereditary properties determining the form independently of the nucleus” (p. 236). Based on an energy usage analysis, Davies, Rieper, and Tuszynski (44) estimated that the total information coding capacity of a typical cell is on the order of 1,000 times that of its genome. Is it reasonable to assume that this enormous excess of information is either redundant or generic? It is clear from the previous discussion that this information cannot be generic. Can it be redundant? As we show below, the answer is no: across phylogeny, information encoded at the architectome scale is both biologically significant and non-redundant.

Morphological diversity anticorrelates with genetic diversity.

If morphology emerged from interactions between genome-encoded macromolecules, one would expect morphological diversity to correlate with genetic diversity. Deep phylogenetic trees (20-22), however, indicate that this is not the case. The great majority of genetic diversity is located in the microbial (bacterial and archaeal) world; indeed it is located in what Danchin (40) has called the “paleome” of genes encoding housekeeping, gene expression, and replication functions (45-47). Microbial morphology, however, does not exhibit high diversity. Microbial adaptability instead depends on the abilities of microbes to incorporate needed functions from a “pangenome” accessed through HGT (46, 48) and to cooperate with metabolically and genomically heterologous cells to facilitate community survival (49-52). As Robbins, Krishtalka, and Wooley (48) point out, the behavior of individual microbial cells is primarily biochemical, not mechanical. Complex morphology is not required to support this lifestyle. Microbial biofilms and migratory aggregates do exhibit mechanical behavior, and have more diverse morphology.

Genetic diversity among eukaryotes is almost negligible compared to that of prokaryotes (see especially (22)). While evidence for HGT among eukaryotes is increasing (53), its effect on total diversity remains small. Even as single cells, eukaryotes interact with their environments mechanically, and they exhibit enormous morphological diversity. The prokaryotic-eukaryotic split can, therefore, be seen as a divergence between a low morphological diversity/high genetic diversity lifestyle and a high morphological diversity/low genetic diversity lifestyle as shown in Fig. 4. This divergence is just the opposite of what a purely-emergence based model of morphological diversity would predict. It suggests that prokaryotic evolution is dominated by genome-scale changes, while eukaryotic evolution is dominated by architectome-scale changes.

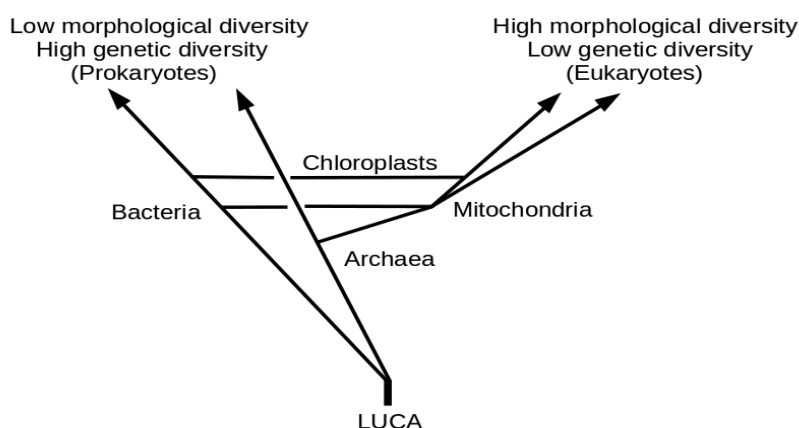


Fig. 4: Simplified phylogeny showing divergence between genetic and morphological diversity, and hence between genome-dominated and architectome-dominated evolutionary processes.

The decoupling of genome from architectome is illustrated at the single-lineage scale by the differentiated cells of multicellular organisms, particularly mammals, in which a single genome is compatible with extreme morphological diversity. Striking evidence for the opposite decoupling, of architectome from genome, is provided by recent results of Nishimura *et al.* (54), who show that a single clonal strain of planaria (*Dugesia*) maintain a fixed morphology and behavioral repertoire over 20 years of asexual reproduction during which non-synonymous codon substitutions accumulated in 74% of predicted genes. More broadly, planaria manage to maintain an extremely robust, invariant morphology despite hundreds of millions of years of clonal reproduction – a process that bypasses Weissman’s barrier and thus accumulates somatic mutations (6). While this result remains to be fully analyzed in planaria or extended to other species, it suggests that the architectome can in at least some cases be enormously resistant to genome-scale variation.

Genome-encoded information can alter, but does not generate, architectome-level information.

Information encoded by the architectome can be viewed as redundant with information encoded by the genome only if it can be completely replaced by genome-encoded information. As noted earlier, strict redundancy between a nonlinear dynamical system and its boundary conditions is forbidden mathematically (31). Biological systems inevitably employ feedback to maintain homeostasis (e.g. (5)); hence their dynamics are nonlinear. At least some architectome-level information cannot, therefore, be redundant. In the language of the “genome as program” metaphor, the architectome provides a non-trivial “operating system” that executes the instructions encoded by the genome. In the limiting cases (in bacteria) of whole-genome replacement by a heterologous (55) or even fully-synthetic genome (56), correct expression of the introduced genome requires a compatible recipient proteome in the context of a compatible cellular structure. Similarly in multicellular eukaryotes, nuclear transplantation requires a compatible cytoplasm; indeed the cytoplasm must be capable of reprogramming the nucleus to totipotency if it is derived from a differentiated cell (57, 58). Experiments with synthetic cell-free systems require spatial organization – and hence architectural information – that is not encoded by the genome (59); similarly, whole-cell metabolic modeling requires a priori modularization assumptions that effectively impose an overall functional architecture on the modeled cell (60). Polyextremophiles such as *Deinococcus radiodurans* withstand doses of ionizing radiation sufficient to create hundreds of double-strand breaks in the genome, effectively destroying its functional integrity (61, 62); cytoplasmic filtrates of *D. radiodurans* are able to protect *E. coli* and even human cells from similar high-dosage radiation (63). No instance of the reverse case, of genomic memory being able to bridge an event that destroys the functional integrity of the CCM system, is known. For example, the

genome cannot rescue ciliates in which a patch of experimentally-reversed cilia pushes food away from the mouth: they starve, and a normal genome cannot rescue them (64).

The process of cellular reproduction (i.e., of cell division followed by regeneration of missing structures by both daughter cells) provides the oldest and most biologically-essential instance of both the action and the inheritance of the architectome. While it is best characterized in large eukaryotic cells, both the major steps and the mechanisms in prokaryotic cells appear to be similar (29). In eukaryotes, the mechanical processes of cell division require the dynamic spatial balancing of contractive and resistive forces implemented by proteins anchored to or associated with the submembrane cortex (65-68). This process is not rigidly stereotyped, but responsive to environmental conditions as demonstrated by its response to the application of external forces (66). Successful cell division preserves the spatial association between cytoskeletal proteins and the cortex, effectively replicating it into each daughter cell. Organizing structures such as centrioles are similarly replicated (69). As the cytoskeleton is also specifically associated with receptor- and channel-enriched membrane raft structures (70-72), it can be assumed that the spatial organization of these structures is also preserved in daughter cells. Active cytoskeleton-mediated subcellular localization of mRNAs in eukaryotes (73, 74) and probably also in prokaryotes (75, 76) provides additional evidence for functionally significant, long-term spatial memories, i.e. for the architectome as an independent component of intergenerational biological memory.

Multicellular morphology and behavior depend on cellular morphology and behavior, and hence on architectome-encoded information, even in the case of microbial communities (77). Homeotic transformations, the most obvious examples of direct genetic impacts on morphology, replicate, move or remove anatomical structures but do not create novel structures. In sexually-reproducing multicellular organisms, architectome-encoded information is squeezed through a zygotic bottleneck. Spatially-segregated germ “granules” (“P-granules”) or germ-line specific cytoplasmic factors including small RNAs (78, 79) are zygotic carriers of architectural information in many but not all animals. Spatial segregation of membrane-bound proton pumps and hence transmembrane proton flux similarly carries information on left-right patterning in *Xenopus* and at least some other vertebrate zygotes (80, 81). As discussed below, however, the investigation of architectome-level inheritance is more tractable in unicellular systems in which reproduction involves regeneration of missing cellular structures from a parent half-cell or in regeneration-competent multicellular organisms.

Architectome-level variants are heritable.

The above considerations suggest that epigenetic inheritance operates not just at the relatively well-understood levels of the transcriptome or proteome (82, 83), but also at the level of the architectome. Since the pioneering work of

Beisson and Sonneborn (64) demonstrating inheritance of experimentally-induced alterations of cortical pattern in *Paramecium*, evidence for architectome-level epigenetics has steadily increased (1). Nelson, Frankel, and Jenkins (84), for example, showed that the polar orientation (“handedness”) of cortical structures with respect to the anterior-posterior axis in *Tetrahymena* was inherited without genetic change. As these authors point out, this result provides an intracellular example of the polar coordinate or “clock face” model of insect and amphibian limb regeneration (85), which is hypothesized to be implemented at the multicellular level by combinatorial Hox gene expression in vertebrates (86). Abenza *et al.* (87) showed that genetically “straight” and “curved” *S. pombe* divide to produce straight and curved progeny stochastically, indicating that cytoskeletal organization could phenocopy variants in cytoskeletal protein structure. Plattner (88) showed that in the classical *Paramecia* system, a component of membrane-encoded architectural information is the geometric arrangement of Ca²⁺ channels (see also below).

The idea that morphological outcomes in multicellular organisms are encoded by large-scale patterns of “positional information” has a long history (89-92). How these patterns are implemented, however, has remained unclear, with Modern Synthesis assumptions consistently pointing towards differential gene expression as the *source* of spatial patterning. Recent experimental results, however, show that this assumption of pattern emergence from the genome is incorrect in at least some multicellular systems. Livshits *et al.* (93), for example, show that the morphology of *Hydra* regenerates depends directly on the configuration of the actomyosin cytoskeleton, which is mechanically continuous at the supracellular level, in the regenerating fragment. Recent studies (94, 95) similarly show that a brief perturbation of gap junction-mediated intercellular communication following amputation in planaria (*Dugesia*) permanently alters regenerative outcomes (12). As shown in Fig. 5, pharyngeal fragments of wild-type (WT) planaria treated briefly post-amputation with octanol to inhibit gap junction communication regenerate as two-headed animals (94). Remarkably, in future rounds of regeneration in plain water, over many months, these animals continue to regenerate two-headed forms even though the octanol is gone from the tissues in 24 hours, the ectopic head tissues are removed in each round (leaving an anatomically normal middle), and the genomic sequence is unaltered. A transient change in the topology of the bioelectric circuit permanently changes the target morphology of these animals – the anatomy to which they regenerate upon damage. Recent work (96) revealed that their target morphology *in vivo* is encoded via global bioelectric states of the body and can diverge from the current anatomy. Worms that are 1-headed and normal at the anatomical, histological, and transcriptional levels can be made to harbour a pattern memory encoding a 2-head worm state; this memory is now directly detectable via voltage dyes and becomes active only if the worm is cut, at which point it can instruct the creation of a 2-headed regenerate from a seemingly normal 1-head parent worm. These data reveal the profound distance between the genome and the resulting anatomy. Not only is morphological pattern not directly encoded in the genome, a rich computational layer exists between them that bears

information that can be dynamically re-written. Moreover, such altered pattern memory is stable across planarians' most common reproductive mode – fission. Importantly, the double-headed state can be re-set back to normal by targeting the bioelectric circuit responsible for anterior-posterior patterning, revealing a molecularly-tractable model of bi-stable, large-scale pattern memory that may facilitate studies of long-neglected phenomena such as trophic memory in deer antler regeneration (reviewed in (97)).

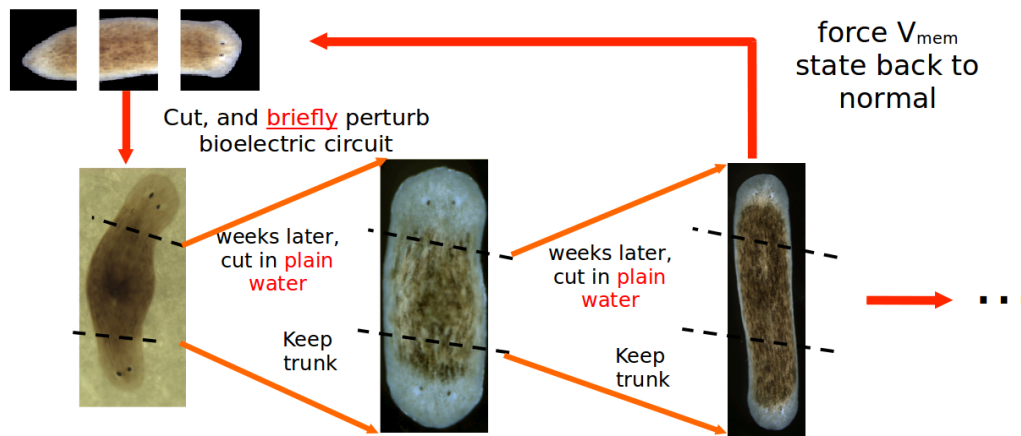


Fig. 5: Non-genomic inheritance of pattern memory in planaria. Pharyngeal fragments of wild-type (WT) planaria treated briefly post-amputation with octanol to inhibit electrical synapses (gap junction communication) regenerate two-headed animals that, when cut again in water, continue to regenerate two-headed animals in perpetuity. The two-headed phenotype can be rescued back to the single-headed form by experimentally re-setting the bioelectric circuit back to wild-type state. This stable change to the animals' target morphology is achieved without editing the genomic sequence or transgenesis.

While technical challenges have limited direct studies of architectome-level inheritance in whole animals, evidence supporting a role for architectome-scale information in patterning from individual organs to whole bodyplans is plentiful (11, 13, 98, 99). From requirements for neural activity for regenerative competence in both neurons (100) and non-neural tissues (101), to the control of regenerative and developmental anatomy by endogenous patterns of resting potentials (102-104) to the association of transmembrane ionic currents, particularly of Ca^{2+} , with morphogenesis in yeast (105, 106) and plant cells (107-109), bioelectric implementation of architectome-scale pattern memory appears to be ubiquitous in multicellular organisms. Bioelectricity provides, moreover, an experimental route into architectome-scale encodings that complements ultrastructural analysis and is more tractable than large-scale metabolomics (98,

110, 111). We suggest that endogenous bioelectricity plays a specific role in the CCM system: that of providing an error-correction mechanism for CCM-encoded memories.

Bioelectricity provides an error-correcting core for target morphology.

Memories require error correction.

Memories accumulate errors; hence memory systems require error correction mechanisms. Error correction is ubiquitous in biological systems, from DNA repair to feedback mechanisms for the maintenance of homeostasis to the inferential checks and balances of conscious recollection. Without such mechanisms mutations accumulate exponentially, metabolism spins out of control, and higher cognition becomes impossible.

Within any given species, memory for morphology is impressively accurate: individuals born in different environments nonetheless develop recognizable, species-appropriate shapes, sizes and internal anatomies. Limbs and other organs capable of regeneration reach the correct size and then stop growing. The stereotypical anatomy of a given species, as implemented by all normal offspring in development and repaired during regeneration, is known as its target morphology. Crucially, this is not merely an open-loop emergent system driven by genomic information. Remodeling (such as when a tail transplanted to a flank progressively turns into a limb (112), appropriate and strictly limited regeneration (e.g., of salamander limbs cut at different anterior-posterior positions (113)), and regulative development (114, 115) all require a feedback process that allows the system to create the appropriate target morphology from diverse starting conditions. Errors in these processes produce birth defects, cancers, and other pathologies, all highlighting the central importance of flexible, robust computation in pattern control throughout metazoa.

What explains the error-correction capability of morphological development? Any error-correction mechanism requires the ability to detect system states that qualify as “errors” and to act on the system being regulated to correct them. As shown by Conant and Ashby (116), any such system requires a model, i.e. a memory, of correct system behavior (cf. (117); see (33) for further elaboration of this result for biological systems in particular). An efficient way to represent shape is by a code that is itself spatial. Electric fields and current flows are intrinsically spatial; moreover, ion channels/pumps (and electrical synapses) are a ubiquitous and ancient feature of living cells, not limited to nerve and muscle (14, 118-120). Hence, developmental bioelectricity is a good candidate to provide an error-correcting morphological code.

Ion channel distribution and bioelectric currents are mutually stabilizing.

The desmid alga *Micrasterias* regenerates a strikingly complex geometric structure after each symmetric cell division, and has long been used as a model system in which to study potential CCM-encoded intergenerational memory (43, 121, 122). As shown in Fig. 6, Ca^{2+} currents flow into growing *Micrasterias* half-cells at the locations of maximal growth, as they do in many other algal and fungal systems (107). Ionic currents exit from non-growing areas of the daughter half-cell as well as from the parent, forming a closed loop that preserves the spatial symmetry of the parent morphology at progressively larger scales as the daughter half-cell increases in size.

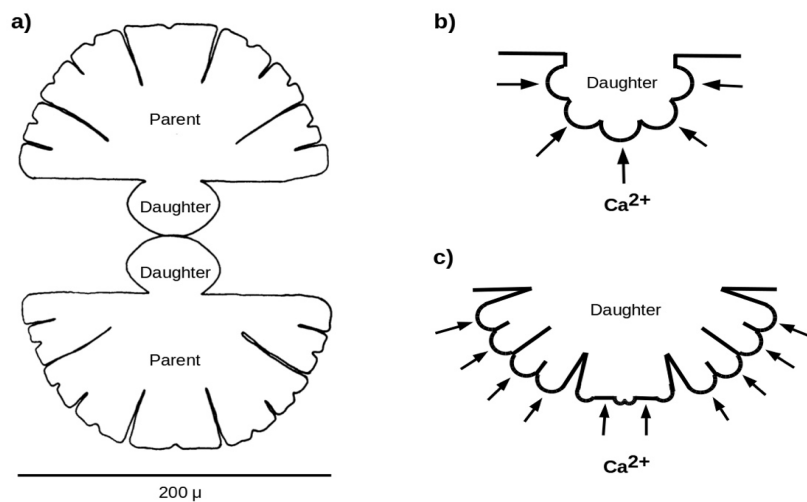


Fig. 6: Symmetric cell division in the desmid alga *Micrasterias*. a) Sketch of planar parent and daughter half-cells immediately following division of the parent cell (adapted from (122), Fig. 2). b) Initial structuring of the daughter cell wall immediately following the state shown in a). Arrows show sites of Ca^{2+} influx as determined by vibrating-probe electrophysiology. c) Subsequent ramification of the structure shown in b); arrows show sites of Ca^{2+} influx. See Meindl (1999), Fig. 1 d-j for high-resolution photomicrographs of this process.

The spatial pattern of Ca^{2+} influx is determined by the spatial pattern of Ca^{2+} channels in the cell membrane, which in turn depends on functional integrity of the actin cytoskeleton (121). Experimental disruption of either Ca^{2+} influx or the actin cytoskeleton disrupts morphogenesis, while microtubule disruption, interestingly, does not. Self-stabilizing reciprocal regulation between Ca^{2+} currents and the actin cytoskeleton has been demonstrated in neurons (123, 124), T cells (125) and apical tips of pollen tubes (126); it is reasonable to

assume that reciprocal Ca^{2+} - actin regulation is similarly self-stabilizing in *Micrasterias*.

While the mechanisms regulating the final size to which daughter *Micrasterias* half-cells grow are not known, results from multiple animal systems implicate bioelectric regulation of limb and organ size as well as shape (19). The bilateral symmetry of mature *Micrasterias* cells enables mechanical tension in the actin cytoskeleton of the parent half-cell to serve as a reference for mechanical tension in the daughter half-cell. It is tempting to speculate that balanced tensile forces (127-129) across the actin network in the mature cell provide the signal that turns Ca^{2+} channels off and hence stops growth-inducing Ca^{2+} influx. Similar arguments have been made about the role of ionic currents in polarization at the cell (130, 131) and organism-wide (102) levels in numerous other species (106, 109).

Bioelectricity enables organism-scale communication.

Macroscopic, organ- or organism-scale electric fields have been characterized in many systems (19) and enable such technologies as EEG and EKG. Static potential differences across body axes are known to regulate both growth and form; for example, altering the bioelectrical connectivity within planarian fragments induces the regeneration of heads with external and internal structures appropriate to other species of flatworm without genome editing (103). Hyperpolarizing a regenerating head in planaria, for example, decreases head size relative to the pharynx (132); mutations of specific ion channels regulate fin size in zebrafish (133), while targeted alterations of resting potential patterns can induce the formation of complete eyes in aberrant locations such as gut, induce the regeneration of complete appendages (134), and regulate the size of the nascent brain (135). The importance of large-scale bioelectric networks for integrating information long distances within the body (not just the brain) was pointed out long ago by Burr in the context of tumorigenesis (136-138). Non-static organism-scale electric fields also, however, appear to play a role in developmental regulation and tumor suppression (139, 140). Chernet, Fields and Levin (141) showed that in *Xenopus* embryos, enhancing or facilitating gap-junction communication between cells on one side of the embryo resulted in differential response to *KRAS*-mediated tumor induction on the opposite side. As shown in Fig. 7, a model that assumes that cells on the left and right sides exchange a “handshaking” signal via an oscillatory electric field quantitatively reproduces the tumor-response observations across a broad range of treatment conditions. Similar cross-body communication via electrical synapses was shown to be critical in determining Left vs. Right identity of the embryonic halves during early left-right patterning (142, 143).

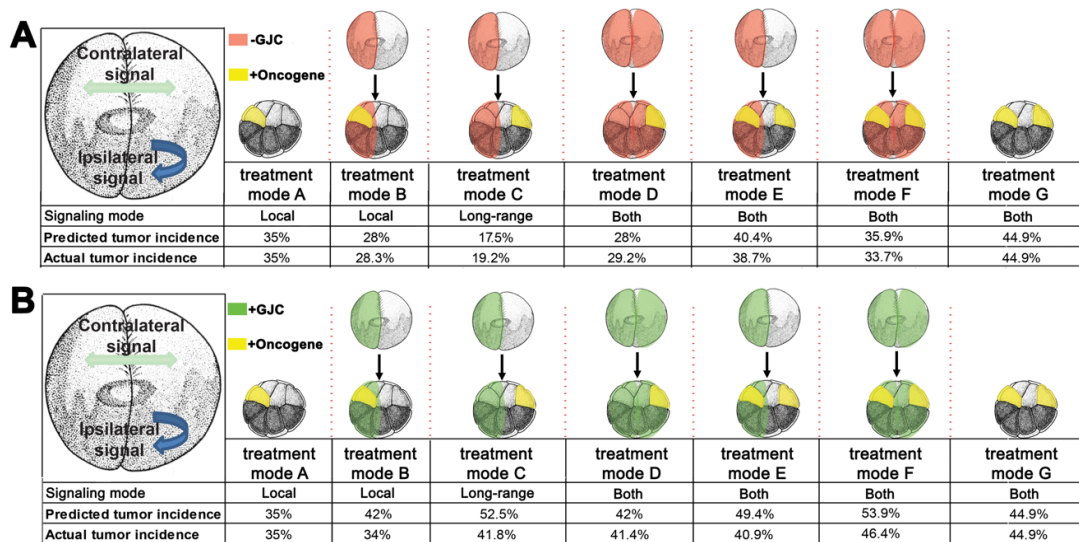


Fig. 7: The results of experiments with *Xenopus* embryos in which gap-junction communication (GJC) was either inhibited (top panel) or enhanced (bottom panel) in the context of tumor-like structures induced by injecting mRNA encoding a KRAS mutant in various locations relative to the gap junctional inhibiting protein. The response to KRAS transformation assayed could be explained by assuming that the two halves of the embryo exchange an oscillatory “handshaking” signal that inhibits cell division (adapted from (141)).

From heartbeat to the cortico-thalamic oscillations that regulate wakefulness and conscious cognition (144), oscillatory bioelectric fields are ubiquitously involved in functional regulation. As noted earlier, neural activity is typically required for regeneration. The results of Chernet, Fields, and Levin (141) suggest that oscillatory electric fields “kick-started” by zygotic asymmetries in membrane ion-channel distribution, and hence presumably in cytoskeletal organization, may implement organism-scale communication that enables balanced, symmetry-preserving morphogenesis in multicellular systems more generally. Recent work has revealed the sources of developmentally-relevant voltage gradients, through the characterization of birth defects induced by channelopathies (145, 146) and the realization that ion channel drugs are not only teratogens (147) but also a potential toolkit of morphochemicals for stem cell biology (148, 149), regenerative medicine (150), and cancer (151, 152). Moreover, transduction mechanisms (11) and downstream transcriptional targets of bioelectric state change (153) have been identified. But it is important to note that bioelectric signaling, as in the CNS, is not just yet another pathway for control of cellular behaviors (154): it is also a system for integrating individual cell dynamics toward large-scale anatomical outcomes.

Bioelectric dysregulation results in morphological dysregulation.

Cancers are commonly thought of as diseases of genetic dysregulation. Goding, Pei, and Lu (155) have suggested that genetic dysregulation in cancer may, however, be an effect of micro-environment dependent nuclear reprogramming by an effectively “transformed” cytoplasm, echoing classical thought about cancer as a problem of pattern disorganization more than of irrevocable damage in individual cells (156-158). If bioelectric “handshaking” regulates growth and stabilizes cell state, one would expect disruptions of bioelectric signaling to dysregulate cellular ion concentrations with subsequent effects on cytoskeletal organization, gene expression, metabolism, and signal transduction (159). Disruption of bioelectric signaling has in fact been demonstrated as an early step in cancer (160, 161), and experimental control of resting potential gradients *in vivo* has been shown to induce metastasis (162) or reverse/prevent tumorigenesis (163, 164). The importance of bioelectric signaling in cells’ defection from the correct morphogenetic plan and toward tumorigenesis is closely tied to their role as one of the mediators of large-scale organizational influences that normally orchestrate pattern regulation.

Endogenous bioelectric gradients have long been thought of as a kind of scaffold (165), similar in a sense to the chemical gradient Hox codes that have been the focus of much research in developmental biology (166). More recent work has hypothesized that these not only serve as direct prepatterns (146, 167), but also implement a computational network that implements global pattern memories (19) using an ancient set of dynamics that evolution later optimized for speed as memories in the brain (168).

How are the spatial symmetries that could serve as a bioelectric “model” of the body implemented? The hierarchical organization of cells into neighborhoods, tissues, organs, and systems suggests that spatial symmetries at multiple scales must be coordinated in a self-stabilizing way. Constructive and destructive interference between oscillations with different temporal and spatial frequencies provides a potential mechanism for such coordination, one that is employed extensively for multi-process coordination in the brain ((169-171)). Interference-based codes naturally amplify coherent signals and damp incoherent signals, indicating that they are naturally error-correcting; the availability of biophysical modeling platforms (172) now makes it tractable to begin to develop testable, quantitative models of large-scale biophysics with the desired computational, error-correcting, and self-organizing properties.

The genome is a resource for, not the source of, the architectome.

The “genetic program” encodes components for assembly, not morphology.

If morphology does not entirely “emerge” from genetics, what is the correct relationship between the two? The experimental results and theoretical considerations reviewed here suggest that morphology is encoded by the

architectome at the cellular level and by cell-cell interactions, including bioelectric signaling, at the supracellular level. If this is the case, the active “agent” of gene regulation is the cytoplasm. The purpose of gene expression is, moreover, clear: it is to obtain the components needed for ongoing cellular processes. From this perspective, the genome is not the “control center” of the cell, but is rather a resource: a memory for component structure from which components for assembly can be obtained.

Conceptualizing the genome as a memory instead of a controller recasts signal transduction *through* the cytoplasm as computation *within* the cytoplasm. Coupling between signal-transduction pathways becomes message passing between computational modules. The “meaning” of a signal is its meaning for the cytoplasm. Hoffmeyer's (173) suggestion that the endoplasmic reticulum be viewed as a “surface” on which information can be encoded – a “working memory” for the cytoplasm – makes sense in this regard.

Morphological diversity expresses architectome-scale evolutionary change.

Viewing the cell and hence the CCM system as the primary locus of biological memory leads also to a reconceptualization of cell division and hence of evolution. The cell is no longer merely a “vehicle” for the genome; the genome is replicated because the cell needs it, not vice-versa. Genetic changes provide modified components that the cell may or may not be able to use; genetic variation in regulatory regions alters the response of the genome to resource requests from the cell. The internal organization of the cell is, however, also open to variation independently of the genome. As this organization becomes more complex, such variation is more likely to feed back onto the genome as epigenetic reprogramming. The concept of inclusive inheritance (174, 175) begins to capture the expanded complexity of an evolutionary system in which variation is bidirectionally coupled across spatial and organizational scales.

The fundamental division between prokaryotes and eukaryotes becomes clearer when evolution itself is viewed as a multi-scale process. Endosymbiosis and the invention of internal membranes fundamentally increased the computing power of the cytoplasm. It is this increase in computing power, we suggest, that enabled the eukaryotic exploration of morphological diversity, an exploration of which prokaryotes are computationally incapable.

Conclusion

We have reviewed evidence supporting three fundamental conclusions that diverge from the mainstream gene-focused paradigm:

- The widespread idea that the genome encodes all or even most biological

information is wrong. The genome is one of many multi-generational biological memories, all of which are necessary for living systems to function.

- All of life shares a continuous membrane and cytoplasm. The external environment is effectively an interstitial space through which messages are passed using bioelectric as well as biophysical and biochemical codes.
- Biological memory cannot be understood at any isolated scale.

We suggest that the next generation of scientists should complement the Modern Synthesis with a different perspective that emphasizes an information- and cognitive-science perspective, a focus on large-scale phenotypes such as anatomical pattern, and biophysical mechanisms as tractable loci for intervention. The couplings between biological memory structures at multiple scales constitute a message-passing network of extraordinary complexity, the outlines of which we are only beginning to grasp. This complexity is balanced by the observation that by modulating bioelectric circuits, complex, coordinated, and self-limiting changes in large-scale anatomy can be triggered without having to specify micro-state information at the level of individual cells. Thus, exploiting highly modular top-down control afforded by CCM-implemented memory systems may facilitate rational control of patterning in biomedical and artificial life contexts. Understanding this network both structurally and functionally will enable, we suggest, significant advances not only in developmental biology but also in clinical applications. In addition to systems-level computational modeling (34, 172, 177), next steps include broader investigation of the origins, distribution, and non-neural function of neurotransmitter systems (178, 179) and mapping ion channel distributions in the membranes of zygotes across a broad phylogenetic range to identify information that could be passed on to their progeny as patterns of bioelectric states (180-182). The increased control over growth and form afforded by the ability to address layers of biological control beyond the genome will lead to transformative new capabilities in synthetic bioengineering and regenerative medicine.

Notes

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References

1. Harold FM. Molecules into cells: specifying spatial architecture. *Microbiology and molecular biology reviews* : MMBR. 2005;69(4):544-64.
2. Basu B, Bruedner M. Cilia: Multifunctional Organelles at the Center of Vertebrate Left-Right Asymmetry. *Ciliary Function in Mammalian Development*. 2008;85:151-74.
3. Brown NA, Wolpert L. The development of handedness in left/right asymmetry. *Development*. 1990;109(1):1-9.
4. Vandenberg LN, Lemire JM, Levin M. It's never too early to get it Right: A conserved role for the cytoskeleton in left-right asymmetry. *Commun Integr Biol*. 2013;6(6):e27155.
5. Ashby WR. *An introduction to cybernetics*. London, U.K.: Chapman & Hall; 1956.
6. Neuhof M, Levin M, Rechavi O. Vertically- and horizontally-transmitted memories - the fading boundaries between regeneration and inheritance in planaria. *Biol Open*. 2016;5(9):1177-88.
7. Zuckerkandl E, Pauling L. Evolutionary Divergence and Convergence in Proteins. In: Bryson V, Vogel HJ, editors. *Evolving genes and proteins*. New York, NY: Academic Press; 1965. p. 97-166.
8. Dawkins R. Replicators and vehicles. In: Brandon RN, Burian RM, editors. *Genes, Organisms, Populations: Controversies Over the Units of Selection*. Cambridge, MA: The MIT Press; 1984. p. 161-80.
9. Way JC, Collins JJ, Keasling JD, Silver PA. Integrating biological redesign: where synthetic biology came from and where it needs to go. *Cell*. 2014;157(1):151-61.
10. Bates E. Ion Channels in Development and Cancer. *Annu Rev Cell Dev Biol*. 2015;31:231-47.
11. Levin M. Molecular bioelectricity: how endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo. *Mol Biol Cell*. 2014;25(24):3835-50.
12. Levin M. Endogenous bioelectrical networks store non-genetic patterning information during development and regeneration. *J Physiol*. 2014;592(11):2295-305.
13. Sullivan KG, Emmons-Bell M, Levin M. Physiological inputs regulate species-specific anatomy during embryogenesis and regeneration. *Commun Integr Biol*. 2016;9(4):e1192733.
14. Funk HR. Ion Gradients in Tissue and Organ Biology. *Biological Systems: Open Access*. 2012;02(01).
15. Di Martino A, Fair DA, Kelly C, Satterthwaite TD, Castellanos FX, Thomason ME, et al. Unraveling the miswired connectome: a developmental perspective. *Neuron*. 2014;83(6):1335-53.
16. Dehaene-Lambertz G, Spelke ES. The Infancy of the Human Brain. *Neuron*. 2015;88(1):93-109.
17. Vertes PE, Bullmore ET. Annual research review: Growth connectomics--the organization and reorganization of brain networks during normal and abnormal development. *J Child Psychol Psychiatry*. 2015;56(3):299-320.
18. Levin M. The wisdom of the body: future techniques and approaches to morphogenetic fields in regenerative medicine, developmental biology and cancer. *Regen Med*. 2011;6(6):667-73.

19. Pezzulo G, Levin M. Re-membering the body: applications of computational neuroscience to the top-down control of regeneration of limbs and other complex organs. *Integr Biol (Camb)*. 2015;7(12):1487-517.
20. McInerney JO, O'Connell MJ, Pisani D. The hybrid nature of the Eukaryota and a consilient view of life on Earth. *Nat Rev Microbiol*. 2014;12(6):449-55.
21. Forterre P. The universal tree of life: an update. *Front Microbiol*. 2015;6:717.
22. Hug LA, Baker BJ, Anantharaman K, Brown CT, Probst AJ, Castelle CJ, et al. A new view of the tree of life. *Nat Microbiol*. 2016;1:16048.
23. Lesch BJ, Page DC. Genetics of germ cell development. *Nat Rev Genet*. 2012;13(11):781-94.
24. Torday JS. The cell as the mechanistic basis for evolution. *Wiley Interdiscip Rev Syst Biol Med*. 2015;7(5):275-84.
25. Woese CR. On the evolution of cells. *Proc Natl Acad Sci U S A*. 2002;99(13):8742-7.
26. Martin WF, Garg S, Zimorski V. Endosymbiotic theories for eukaryote origin. *Philos Trans R Soc Lond B Biol Sci*. 2015;370(1678):20140330.
27. Cavalier-Smith T. Membrane heredity and early chloroplast evolution. *Trends Plant Sci*. 2000;5(4):174-82.
28. Erickson HP. Evolution of the cytoskeleton. *Bioessays*. 2007;29(7):668-77.
29. Wickstead B, Gull K. The evolution of the cytoskeleton. *J Cell Biol*. 2011;194(4):513-25.
30. Polanyi M. Life's irreducible structure. Live mechanisms and information in DNA are boundary conditions with a sequence of boundaries above them. *Science*. 1968;160(3834):1308-12.
31. Rosen R. On information and complexity. In: Casti JL, Karlqvist A, editors. *Complexity, Language, and Life: Mathematical Approaches*. Berlin, Germany: Springer-Verlag; 1986. p. 174-96.
32. Pearl J. Probabilistic reasoning in intelligent systems: networks of plausible inference. San Francisco, CA: Morgan Kaufmann Publishers Inc.; 1988.
33. Friston K. Life as we know it. *J R Soc Interface*. 2013;10(86):20130475.
34. Friston K, Levin M, Sengupta B, Pezzulo G. Knowing one's place: a free-energy approach to pattern regulation. *J R Soc Interface*. 2015;12(105):20141383.
35. Cereghino JL, Cregg JM. Heterologous protein expression in the methylotrophic yeast *Pichia pastoris*. *FEMS Microbiol Rev*. 2000;24(1):45-66.
36. Barnes LA. The Fine-Tuning of the Universe for Intelligent Life. *Publications of the Astronomical Society of Australia*. 2012;29(4):529-64.
37. Ryan AJ. Emergence is coupled to scope, not level. *Complexity*. 2007;13(2):67-77.
38. Butterfield J. Emergence, Reduction and Supervenience: A Varied Landscape. *Foundations of Physics*. 2011;41(6):920-59.
39. Licata I, Minati G. Emergence, Computation and the Freedom Degree Loss Information Principle in Complex Systems. *Foundations of Science*. 2016:1-19.
40. Danchin A. Bacteria as computers making computers. *FEMS Microbiol Rev*. 2009;33(1):3-26.
41. Hutchison CA, 3rd, Chuang RY, Noskov VN, Assad-Garcia N, Deerinck TJ, Ellisman MH, et al. Design and synthesis of a minimal bacterial genome. *Science*. 2016;351(6280):aad6253.
42. Pezzulo G, Levin M. Top-down models in biology: explanation and control of complex living systems above the molecular level. *J R Soc Interface*. 2016;13(124):20160555.
43. Waris H. Cytophysiological Studies on *Micrasterias* II. The Cytoplasmic Framework and its Mutation. *Physiologia Plantarum*. 1950;3(3):236-46.

44. Davies PC, Rieper E, Tuszynski JA. Self-organization and entropy reduction in a living cell. *Biosystems*. 2013;111(1):1-10.
45. Dagan T, Martin W. The tree of one percent. *Genome Biol*. 2006;7(10):118.
46. Doolittle WF, Bapteste E. Pattern pluralism and the Tree of Life hypothesis. *Proc Natl Acad Sci U S A*. 2007;104(7):2043-9.
47. Koonin EV. Horizontal gene transfer: essentiality and evolvability in prokaryotes, and roles in evolutionary transitions. *F1000Res*. 2016;5.
48. Robbins RJ, Krishtalka L, Wooley JC. Advances in biodiversity: metagenomics and the unveiling of biological dark matter. *Stand Genomic Sci*. 2016;11(1):69.
49. West SA, Griffin AS, Gardner A, Diggle SP. Social evolution theory for microorganisms. *Nat Rev Microbiol*. 2006;4(8):597-607.
50. Mitri S, Foster KR. The genotypic view of social interactions in microbial communities. *Annu Rev Genet*. 2013;47:247-73.
51. Gloag ES, Turnbull L, Whitchurch CB. Bacterial stigmergy: an organising principle of multicellular collective behaviours of bacteria. *Scientifica (Cairo)*. 2015;2015:387342.
52. West SA, Cooper GA. Division of labour in microorganisms: an evolutionary perspective. *Nat Rev Microbiol*. 2016;14(11):716-23.
53. Shapiro BJ, Leducq JB, Mallet J. What Is Speciation? *PLoS Genet*. 2016;12(3):e1005860.
54. Nishimura O, Hosoda K, Kawaguchi E, Yazawa S, Hayashi T, Inoue T, et al. Unusually Large Number of Mutations in Asexually Reproducing Clonal Planarian *Dugesia japonica*. *PLoS One*. 2015;10(11):e0143525.
55. Lartigue C, Glass JL, Alperovich N, Pieper R, Parmar PP, Hutchison CA, 3rd, et al. Genome transplantation in bacteria: changing one species to another. *Science*. 2007;317(5838):632-8.
56. Gibson DG, Glass JL, Lartigue C, Noskov VN, Chuang RY, Algire MA, et al. Creation of a bacterial cell controlled by a chemically synthesized genome. *Science*. 2010;329(5987):52-6.
57. Gurdon JB. The egg and the nucleus: a battle for supremacy. *Development*. 2013;140(12):2449-56.
58. Halley-Stott RP, Pasque V, Gurdon JB. Nuclear reprogramming. *Development*. 2013;140(12):2468-71.
59. Hodgman CE, Jewett MC. Cell-free synthetic biology: thinking outside the cell. *Metab Eng*. 2012;14(3):261-9.
60. Karr JR, Sanghvi JC, Macklin DN, Gutschow MV, Jacobs JM, Bolival B, Jr., et al. A whole-cell computational model predicts phenotype from genotype. *Cell*. 2012;150(2):389-401.
61. Krisko A, Radman M. Biology of extreme radiation resistance: the way of *Deinococcus radiodurans*. *Cold Spring Harb Perspect Biol*. 2013;5(7):a012765.
62. Pavlopoulou A, Savva GD, Louka M, Bagos PG, Vorgias CE, Michalopoulos I, et al. Unraveling the mechanisms of extreme radioresistance in prokaryotes: Lessons from nature. *Mutat Res Rev Mutat Res*. 2016;767:92-107.
63. Daly MJ, Gaidamakova EK, Matrosova VY, Kiang JG, Fukumoto R, Lee DY, et al. Small-molecule antioxidant proteome-shields in *Deinococcus radiodurans*. *PLoS One*. 2010;5(9):e12570.
64. Beisson J, Sonneborn TM. Cytoplasmic Inheritance of the Organization of the Cell Cortex in *Paramecium Aurelia*. *Proc Natl Acad Sci U S A*. 1965;53(2):275-82.
65. Zhang W, Robinson DN. Balance of actively generated contractile and resistive forces controls cytokinesis dynamics. *Proc Natl Acad Sci U S A*. 2005;102(20):7186-91.
66. Effler JC, Kee YS, Berk JM, Tran MN, Iglesias PA, Robinson DN. Mitosis-

- specific mechanosensing and contractile-protein redistribution control cell shape. *Curr Biol*. 2006;16(19):1962-7.
67. Pollard TD, Cooper JA. Actin, a central player in cell shape and movement. *Science*. 2009;326(5957):1208-12.
 68. Pritchard RH, Huang YY, Terentjev EM. Mechanics of biological networks: from the cell cytoskeleton to connective tissue. *Soft Matter*. 2014;10(12):1864-84.
 69. Meunier A, Spassky N. Centriole continuity: out with the new, in with the old. *Curr Opin Cell Biol*. 2016;38:60-7.
 70. Cowin P, Burke B. Cytoskeleton-membrane interactions. *Curr Opin Cell Biol*. 1996;8(1):56-65.
 71. Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol*. 2000;1(1):31-9.
 72. Chichili GR, Rodgers W. Cytoskeleton-membrane interactions in membrane raft structure. *Cellular and molecular life sciences : CMLS*. 2009;66(14):2319-28.
 73. Holt CE, Bullock SL. Subcellular mRNA localization in animal cells and why it matters. *Science*. 2009;326(5957):1212-6.
 74. Jung H, Gkogkas CG, Sonenberg N, Holt CE. Remote control of gene function by local translation. *Cell*. 2014;157(1):26-40.
 75. Keiler KC. RNA localization in bacteria. *Curr Opin Microbiol*. 2011;14(2):155-9.
 76. Buskila AA, Kannaiah S, Amster-Choder O. RNA localization in bacteria. *RNA Biol*. 2014;11(8):1051-60.
 77. Munoz-Dorado J, Marcos-Torres FJ, Garcia-Bravo E, Moraleda-Munoz A, Perez J. Myxobacteria: Moving, Killing, Feeding, and Surviving Together. *Front Microbiol*. 2016;7:781.
 78. Ouellet J, Barral Y. Organelle segregation during mitosis: lessons from asymmetrically dividing cells. *J Cell Biol*. 2012;196(3):305-13.
 79. Strome S, Updike D. Specifying and protecting germ cell fate. *Nat Rev Mol Cell Biol*. 2015;16(7):406-16.
 80. Levin M, Thorlin T, Robinson KR, Nogi T, Mercola M. Asymmetries in H⁺/K⁺-ATPase and cell membrane potentials comprise a very early step in left-right patterning. *Cell*. 2002;111(1):77-89.
 81. Adams DS, Robinson KR, Fukumoto T, Yuan S, Albertson RC, Yelick P, et al. Early, H⁺-V-ATPase-dependent proton flux is necessary for consistent left-right patterning of non-mammalian vertebrates. *Development*. 2006;133(9):1657-71.
 82. Cunliffe VT. Experience-sensitive epigenetic mechanisms, developmental plasticity, and the biological embedding of chronic disease risk. *Wiley Interdiscip Rev Syst Biol Med*. 2015;7(2):53-71.
 83. Moore DS. Behavioral epigenetics. *Wiley Interdiscip Rev Syst Biol Med*. 2017;9(1).
 84. Nelsen EM, Frankel J, Jenkins LM. Non-genic inheritance of cellular handedness. *Development*. 1989;105(3):447-56.
 85. French V, Bryant PJ, Bryant SV. Pattern regulation in epimorphic fields. *Science*. 1976;193(4257):969-81.
 86. McCusker C, Bryant SV, Gardiner DM. The axolotl limb blastema: cellular and molecular mechanisms driving blastema formation and limb regeneration in tetrapods. *Regeneration (Oxf)*. 2015;2(2):54-71.
 87. Abenza JF, Chessel A, Raynaud WG, Carazo-Salas RE. Dynamics of cell shape inheritance in fission yeast. *PLoS One*. 2014;9(9):e106959.
 88. Plattner H. Molecular aspects of calcium signalling at the crossroads of unikont and bikont eukaryote evolution--the ciliated protozoan *Paramecium* in focus. *Cell Calcium*. 2015;57(3):174-85.
 89. Thompson DAW. On growth and form. Cambridge, U.K.: Cambridge

University Press; 1942.

90. Turing AM. The Chemical Basis of Morphogenesis. *Philos T Roy Soc B*. 1952;237(641):37-72.
91. Wolpert L. Positional information and the spatial pattern of cellular differentiation. *J Theor Biol*. 1969;25(1):1-47.
92. Gierer A, Meinhardt H. A theory of biological pattern formation. *Kybernetik*. 1972;12(1):30-9.
93. Livshits A, Shani-Zerbib L, Maroudas-Sacks Y, Braun E, Keren K. Structural Inheritance of the Actin Cytoskeletal Organization Determines the Body Axis in Regenerating Hydra. *Cell Rep*. 2017;18(6):1410-21.
94. Oviedo NJ, Morokuma J, Walentek P, Kema IP, Gu MB, Ahn JM, et al. Long-range neural and gap junction protein-mediated cues control polarity during planarian regeneration. *Dev Biol*. 2010;339(1):188-99.
95. Durant F, Morokuma J, Fields C, Williams K, Adams DS, Levin M. Long-Term, Stochastic Editing of Regenerative Anatomy via Targeting Endogenous Bioelectric Gradients. *Biophys J*. 2017;112(10):2231-43.
96. Durant F, Morokuma J, Fields C, Williams K, Adams DS, Levin M. Long-Term, Stochastic Editing of Regenerative Anatomy via Targeting Endogenous Bioelectric Gradients. *Biophys J*. 2017;112(10):2231-43.
97. Lobo D, Solano M, Bubenik GA, Levin M. A linear-encoding model explains the variability of the target morphology in regeneration. *J R Soc Interface*. 2014;11(92):20130918.
98. Levin M. Molecular bioelectricity in developmental biology: new tools and recent discoveries: control of cell behavior and pattern formation by transmembrane potential gradients. *BioEssays*. 2012;34(3):205-17.
99. Mustard J, Levin M. Bioelectrical Mechanisms for Programming Growth and Form: Taming Physiological Networks for Soft Body Robotics. *Soft Robotics*. 2014;1(3):169-91.
100. Abe N, Cavalli V. Nerve injury signaling. *Current opinion in neurobiology*. 2008;18(3):276-83.
101. Kumar A, Brookes JP. Nerve dependence in tissue, organ, and appendage regeneration. *Trends Neurosci*. 2012;35(11):691-9.
102. Beane WS, Morokuma J, Adams DS, Levin M. A chemical genetics approach reveals H,K-ATPase-mediated membrane voltage is required for planarian head regeneration. *Chem Biol*. 2011;18(1):77-89.
103. Emmons-Bell M, Durant F, Hammelman J, Bessonov N, Volpert V, Morokuma J, et al. Gap Junctional Blockade Stochastically Induces Different Species-Specific Head Anatomies in Genetically Wild-Type *Girardia dorotocephala* Flatworms. *Int J Mol Sci*. 2015;16(11):27865-96.
104. Pai VP, Aw S, Shomrat T, Lemire JM, Levin M. Transmembrane voltage potential controls embryonic eye patterning in *Xenopus laevis*. *Development*. 2012;139(2):313-23.
105. Minc N, Chang F. Electrical control of cell polarization in the fission yeast *Schizosaccharomyces pombe*. *Curr Biol*. 2010;20(8):710-6.
106. Haupt A, Campetelli A, Bonazzi D, Piel M, Chang F, Minc N. Electrochemical regulation of budding yeast polarity. *PLoS Biol*. 2014;12(12):e1002029.
107. Weisenseel M, Kicherer RM. Ionic currents as control mechanism in cytomorphogenesis. In: Kiermayer O, editor. *Cytomorphogenesis in plants*. Vienna, Austria: Springer-Verlag Wien; 1981. p. 379-99.
108. Hepler PK, Wayne RO. Calcium and Plant Development. *Annual Review of Plant Physiology and Plant Molecular Biology*. 1985;36(1):397-439.
109. Chang F, Minc N. Electrochemical control of cell and tissue polarity. *Annu Rev Cell Dev Biol*. 2014;30:317-36.
110. Levin M. Reprogramming cells and tissue patterning via bioelectrical

pathways: molecular mechanisms and biomedical opportunities. *Wiley Interdiscip Rev Syst Biol Med*. 2013;5(6):657-76.

111. Tseng A, Levin M. Cracking the bioelectric code: Probing endogenous ionic controls of pattern formation. *Commun Integr Biol*. 2013;6(1):e22595.
112. Farinella-Ferruzza N. The transformation of a tail into limb after xenoplastic transplantation. *Experientia*. 1956;12(8):304-5.
113. Birnbaum KD, Sanchez Alvarado A. Slicing across kingdoms: regeneration in plants and animals. *Cell*. 2008;132(4):697-710.
114. Cooke J. Properties of the primary organization field in the embryo of *Xenopus laevis*. V. Regulation after removal of the head organizer, in normal early gastrulae and in those already possessing a second implanted organizer. *J Embryol Exp Morphol*. 1973;30(2):283-300.
115. Tarkowski AK. Mouse chimaeras developed from fused eggs. *Nature*. 1961;190(4779):857-60.
116. Conant RC, Ross Ashby W. Every good regulator of a system must be a model of that system. *International Journal of Systems Science*. 1970;1(2):89-97.
117. Friston K. The free-energy principle: a unified brain theory? *Nat Rev Neurosci*. 2010;11(2):127-38.
118. Burr HS, Northrop FSC. The electro-dynamic theory of life. *Quarterly Review of Biology*. 1935;10(3):322-33.
119. Lund EJ. *Bioelectric Fields and Growth*. Austin, TX: University of Texas Press; 1947.
120. Mathews AP. Electrical polarity in the hydroids. *American Journal of Physiology--Legacy Content*. 1903;8(4):294-9.
121. Lutz-Meindl U. *Micrasterias as a Model System in Plant Cell Biology*. *Front Plant Sci*. 2016;7:999.
122. Meindl U. *Micrasterias Cells as a Model System for Research on Morphogenesis*. *Microbiological Reviews*. 1993;57(2):415-33.
123. Oertner TG, Matus A. Calcium regulation of actin dynamics in dendritic spines. *Cell Calcium*. 2005;37(5):477-82.
124. Sutherland DJ, Pujic Z, Goodhill GJ. Calcium signaling in axon guidance. *Trends Neurosci*. 2014;37(8):424-32.
125. Joseph N, Reicher B, Barda-Saad M. The calcium feedback loop and T cell activation: how cytoskeleton networks control intracellular calcium flux. *Biochim Biophys Acta*. 2014;1838(2):557-68.
126. Qu X, Jiang Y, Chang M, Liu X, Zhang R, Huang S. Organization and regulation of the actin cytoskeleton in the pollen tube. *Front Plant Sci*. 2014;5:786.
127. Chen CS, Ingber DE. Tensegrity and mechanoregulation: from skeleton to cytoskeleton. *Osteoarthritis Cartilage*. 1999;7(1):81-94.
128. Belousov LV. Mechanically based generative laws of morphogenesis. *Physical biology*. 2008;5(1):015009.
129. Belousov LV, Grabovsky VI. Morphomechanics: goals, basic experiments and models. *Int J Dev Biol*. 2006;50(2-3):81-92.
130. Jaffe LF. The role of ionic currents in establishing developmental pattern. *Philos Trans R Soc Lond B Biol Sci*. 1981;295(1078):553-66.
131. Robinson KR, Jaffe LF. Calcium Gradients and Egg Polarity. *J Cell Biol*. 1976;70(2):A37-A.
132. Beane WS, Morokuma J, Lemire JM, Levin M. Bioelectric signaling regulates head and organ size during planarian regeneration. *Development*. 2013;140(2):313-22.
133. Perathoner S, Daane JM, Henrion U, Seebohm G, Higdon CW, Johnson SL, et al. Bioelectric signaling regulates size in zebrafish fins. *PLoS Genet*. 2014;10(1):e1004080.

134. Adams DS, Tseng AS, Levin M. Light-activation of the Archaelhodopsin H(+)-pump reverses age-dependent loss of vertebrate regeneration: sparking system-level controls in vivo. *Biol Open*. 2013;2(3):306-13.
135. Pai VP, Lemire JM, Pare JF, Lin G, Chen Y, Levin M. Endogenous gradients of resting potential instructively pattern embryonic neural tissue via Notch signaling and regulation of proliferation. *J Neurosci*. 2015;35(10):4366-85.
136. Burr HS. Changes in the field properties of mice with transplanted tumors. *Yale Journal of Biology & Medicine*. 1941;13:783-8.
137. Burr HS, Smith GM, Strong LC. Electrometric Studies of Tumors in Mice Induced by the External Application of Benzpyrene. *The Yale Journal of Biology and Medicine*. 1940;12(6):711-7.
138. Burr HS, Strong LC, Smith GM. Bio-Electric Correlates of Methylcolanthrene-Induced Tumors in Mice. *The Yale Journal of Biology and Medicine*. 1938;10(6):539-44.
139. Burr HS, Northrop FS. Evidence for the Existence of an Electro-Dynamic Field in Living Organisms. *Proc Natl Acad Sci U S A*. 1939;25(6):284-8.
140. Nordenström B. Biologically closed electric circuits: Clinical, experimental and theoretical evidence for an additional circulatory system: Princeton University Press; 1983.
141. Chernet BT, Fields C, Levin M. Long-range gap junctional signaling controls oncogene-mediated tumorigenesis in *Xenopus laevis* embryos. *Front Physiol*. 2014;5:519.
142. Levin M, Mercola M. Gap junction-mediated transfer of left-right patterning signals in the early chick blastoderm is upstream of Shh asymmetry in the node. *Development*. 1999;126:4703-14.
143. Levin M, Mercola M. Gap junctions are involved in the early generation of left-right asymmetry. *Dev Biol*. 1998;203(1):90-105.
144. Baars BJ, Franklin S, Ramsoy TZ. Global workspace dynamics: cortical "binding and propagation" enables conscious contents. *Front Psychol*. 2013;4:200.
145. Masotti A, Uva P, Davis-Keppen L, Basel-Vanagaite L, Cohen L, Pisaneschi E, et al. Keppen-Lubinsky syndrome is caused by mutations in the inwardly rectifying K⁺ channel encoded by KCNJ6. *Am J Hum Genet*. 2015;96(2):295-300.
146. Adams DS, Uzel SG, Akagi J, Wlodkowic D, Andreeva V, Yelick PC, et al. Bioelectric signalling via potassium channels: a mechanism for craniofacial dysmorphogenesis in KCNJ2-associated Andersen-Tawil Syndrome. *J Physiol*. 2016;594(12):3245-70.
147. Hernandez-Diaz S, Levin M. Alteration of bioelectrically-controlled processes in the embryo: a teratogenic mechanism for anticonvulsants. *Reprod Toxicol*. 2014;47:111-4.
148. Sundelacruz S, Levin M, Kaplan DL. Membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells. *PLoS One*. 2008;3(11):e3737.
149. Sundelacruz S, Levin M, Kaplan DL. Depolarization alters phenotype, maintains plasticity of predifferentiated mesenchymal stem cells. *Tissue Eng Part A*. 2013;19(17-18):1889-908.
150. Tseng AS, Beane WS, Lemire JM, Masi A, Levin M. Induction of vertebrate regeneration by a transient sodium current. *J Neurosci*. 2010;30(39):13192-200.
151. Arcangeli A, Becchetti A. *New Trends in Cancer Therapy: Targeting Ion Channels and Transporters*. Pharmaceuticals (Basel). 2010;3(4):1202-24.
152. Wulff H, Castle NA, Pardo LA. Voltage-gated potassium channels as therapeutic targets. *Nat Rev Drug Discov*. 2009;8(12):982-1001.
153. Pai VP, Martyniuk CJ, Echeverri K, Sundelacruz S, Kaplan DL, Levin M. Genome-wide analysis reveals conserved transcriptional responses downstream

of resting potential change in *Xenopus* embryos, axolotl regeneration, and human mesenchymal cell differentiation. *Regeneration (Oxf)*. 2016;3(1):3-25.

154. Sundelacruz S, Levin M, Kaplan DL. Role of membrane potential in the regulation of cell proliferation and differentiation. *Stem Cell Rev*. 2009;5(3):231-46.

155. Goding CR, Pei D, Lu X. Cancer: pathological nuclear reprogramming? *Nat Rev Cancer*. 2014;14(8):568-73.

156. Waddington CH. Cancer and the theory of organisers. *Nature*. 1935;135(606):606-8.

157. Needham J. New Advances in the Chemistry and Biology of Organized Growth: (Section of Pathology). *Proc R Soc Med*. 1936;29(12):1577-626.

158. Rubin H. Cancer as a Dynamic Developmental Disorder. *Cancer Research*. 1985;45(7):2935-42.

159. Chernet B, Levin M. Endogenous Voltage Potentials and the Microenvironment: Bioelectric Signals that Reveal, Induce and Normalize Cancer. *J Clin Exp Oncol*. 2013;Suppl 1.

160. Chernet BT, Levin M. Transmembrane voltage potential is an essential cellular parameter for the detection and control of tumor development in a *Xenopus* model. *Dis Model Mech*. 2013;6(3):595-607.

161. Trosko JE. Gap junctional intercellular communication as a biological "Rosetta stone" in understanding, in a systems biological manner, stem cell behavior, mechanisms of epigenetic toxicology, chemoprevention and chemotherapy. *J Membr Biol*. 2007;218(1-3):93-100.

162. Lobikin M, Chernet B, Lobo D, Levin M. Resting potential, oncogene-induced tumorigenesis, and metastasis: the bioelectric basis of cancer in vivo. *Phys Biol*. 2012;9(6):065002.

163. Chernet BT, Levin M. Transmembrane voltage potential of somatic cells controls oncogene-mediated tumorigenesis at long-range. *Oncotarget*. 2014;5(10):3287-306.

164. Chernet BT, Adams DS, Lobikin M, Levin M. Use of genetically encoded, light-gated ion translocators to control tumorigenesis. *Oncotarget*. 2016;7(15):19575-88.

165. Burr HS. The Meaning of Bio-Electric Potentials. *Yale J Biol Med*. 1944;16(4):353-60.

166. Niehrs C. On growth and form: a Cartesian coordinate system of Wnt and BMP signaling specifies bilaterian body axes. *Development*. 2010;137(6):845-57.

167. Vandenberg LN, Morrie RD, Adams DS. V-ATPase-dependent ectodermal voltage and pH regionalization are required for craniofacial morphogenesis. *Dev Dyn*. 2011;240(8):1889-904.

168. Baluska F, Levin M. On Having No Head: Cognition throughout Biological Systems. *Front Psychol*. 2016;7:902.

169. Buzsaki G, Draguhn A. Neuronal oscillations in cortical networks. *Science*. 2004;304(5679):1926-9.

170. Sejnowski TJ, Paulsen O. Network oscillations: emerging computational principles. *J Neurosci*. 2006;26(6):1673-6.

171. Lisman JE, Jensen O. The theta-gamma neural code. *Neuron*. 2013;77(6):1002-16.

172. Pietak A, Levin M. Exploring Instructive Physiological Signaling with the Bioelectric Tissue Simulation Engine. *Front Bioeng Biotechnol*. 2016;4:55.

173. Hoffmeyer J. The biology of signification. *Perspect Biol Med*. 2000;43(2):252-68.

174. Laland KN, Uller T, Feldman MW, Sterelny K, Müller GB, Moczek A, et al., editors. *The extended evolutionary synthesis: its structure, assumptions and predictions*. *Proc R Soc B*; 2015: The Royal Society.

175. Danchin E, Charmantier A, Champagne FA, Mesoudi A, Pujol B, Blanchet S. Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. *Nat Rev Genet.* 2011;12(7):475-86.
176. Rivera MC, Lake JA. The ring of life provides evidence for a genome fusion origin of eukaryotes. *Nature.* 2004;431(7005):152-5.
177. Brodsky M. Partial Redundancy and Morphological Homeostasis: Reliable Development through Overlapping Mechanisms. *Artif Life.* 2016;22(4):518-36.
178. Roth J, LeRoith D, Shiloach J, Rosenzweig JL, Lesniak MA, Havrankova J. The evolutionary origins of hormones, neurotransmitters, and other extracellular chemical messengers: implications for mammalian biology. *N Engl J Med.* 1982;306(9):523-7.
179. Venter JC, di Porzio U, Robinson DA, Shreeve SM, Lai J, Kerlavage AR, et al. Evolution of neurotransmitter receptor systems. *Progress in neurobiology.* 1988;30(2-3):105-69.
180. Head BP, Patel HH, Insel PA. Interaction of membrane/lipid rafts with the cytoskeleton: impact on signaling and function: membrane/lipid rafts, mediators of cytoskeletal arrangement and cell signaling. *Biochim Biophys Acta.* 2014;1838(2):532-45.
181. Harding AS, Hancock JF. Using plasma membrane nanoclusters to build better signaling circuits. *Trends Cell Biol.* 2008;18(8):364-71.
182. Martens JR, O'Connell K, Tamkun M. Targeting of ion channels to membrane microdomains: localization of KV channels to lipid rafts. *Trends Pharmacol Sci.* 2004;25(1):16-21.