The Membrane Code: A Carrier of Essential Biological Information That Is Not Specified by DNA and Is Inherited Apart from It

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Abstract

According to the most widely held modern version of Darwin's theory, DNA mutations can supply raw materials for morphological evolution because they alter a genetic program that controls embryo development. Yet a genetic program is not sufficient for embryogenesis: biological information outside of DNA is needed to specify the body plan of the embryo and much of its subsequent development. Some of that information is in cell membrane patterns, which contain a two-dimensional code mediated by proteins and carbohydrates. These molecules specify targets for morphogenetic determinants in the cytoplasm, generate endogenous electric fields that provide spatial coordinates for embryo development, regulate intracellular signaling, and participate in cell–cell interactions. Although the individual membrane molecules are at least partly specified by DNA sequences, their two-dimensional patterns are not. Furthermore, membrane patterns can be inherited independently of the DNA. I review some of the evidence for the membrane code and argue that it has important implications for modern evolutionary theory.

Key words: gene regulatory networks, embryogenesis, spatial information, membrane patterns, endogenous electric fields, intracellular signaling, sugar code

Introduction

According to the most common modern version of evolutionary theory, genetic programs encoded in linear sequences of DNA are sufficient to control the development of embryos — from their basic body plans to all aspects of their morphology and physiology. Major evolutionary changes would then depend primarily on changes in genetic programs. Although a few biologists are critical of this view [1–3], some evolutionary developmental biologists have recently argued that interacting transcription factors in gene regulatory networks (GRNs) support it.

For example, Eric H. Davidson writes, "The body plan of an animal, and hence its exact mode of development, is a property of its species and is thus encoded in the genome. Embryonic development is an enormous informational transaction, in

which DNA sequence data generate and guide the system-wide spatial deployment of specific cellular functions. Because development of the body plan is caused by the operation of GRNs, evolutionary change in the body plan is change in GRN structure occurring over deep time" [4].

According to Sean B. Carroll, "Given that development is controlled by GRNs, it follows that the evolution of development and form is due to changes within GRNs… I have presented the case for a genetic theory of morphological evolution that can be condensed into two statements: (1) form evolves largely by altering the expression of functionally conserved proteins; and (2) such changes largely occur through mutations in the *cis*-regulatory regions of mosaically pleiotropic developmental regulatory genes" [5].

On occasion, Davidson and Carroll have both acknowledged that GRNs act within preexisting spatial domains, but they argue that such spatial specification can be neglected and that GRNs are the principal factors in development. Davidson writes that animal embryos "illustrate two features. The less important is the variable specifics of the initial cytoplasmic bases of spatial anisotropy. The other feature is of ultimate importance: This is the common functional endpoint of these very diverse initial stratagems for the spatial indication of future developmental domains. The principle is that whatever the bases of the anisotropies, however they come into being, whatever the cell fates that derive from what they set in train, they end up causing certain maternal transcription factors to be present and active in some spatially defined embryo nuclei, but not in others" [6].

According to Carroll, "Ultimately, the beginning of spatial information in the embryo often traces back to asymmetrically distributed molecules deposited in the egg during its production in the ovary that initiate the formation of the two main axes of the embryo (so the egg did come before the chicken). I'm not going to trace these steps — the important point to know is that the throwing of every switch is set up by preceding events, and that a switch, by turning on its gene in a new pattern, in turn sets up the next set of patterns and events in development" [7].

Yet GRNs cannot differentiate one region of the embryo from another without spatial information that is specified beforehand in the fertilized egg. Evidence for this comes from a wide variety of animals.

The Need for Spatial Information Prior to Localization of Gene Products

The maternal, segmentation, and Hox genes in embryos of the fruit fly *Drosophila melanogaster* comprise a GRN, yet that network depends on the prior establishment of the embryo's first body axis by polarized cytoskeletal

arrays and spatially localized targets already present in the oocyte; those polarizations and localizations, in turn, derive from prior asymmetries inherent in the ovary [8–14].

Spatial information also precedes and directs the GRNs in embryos of the nematode *Caenorhabditis elegans.* The sperm centrosome first establishes an anterior-posterior axis by initiating cytoskeletal changes that produce a polarized distribution of zygotic proteins. These in turn lead to asymmetrical cell divisions and subsequent differentiation [15–17].

In ascidian oocytes, the cortex (the cell membrane plus underlying cytoplasmic and cytoskeletal elements) already contains spatially localized morphogenetic determinants that specify the primary axis of the embryo. Upon fertilization, the sperm centrosome induces cytoskeletal changes that reorganize those determinants and establish the second (dorsal-ventral) axis [18,19].

Oocytes of the frog *Xenopus laevis* also have a primary axis before the sperm enters. The sperm establishes a second axis by aligning a microtubule array in the zygote that directs morphogenetic determinants to the future dorsal side of the embryo [20–22].

In all of these cases, spatial coordinates are established in the embryo before zygotic GRNs become active. Such coordinates provide biological information by specifying domains in the embryo that later differentiate by means of GRNs in progressively finer detail. Spatial information can be mediated by polarized cytoskeletal arrays, which in some embryos are reorganized by the sperm upon fertilization. Other spatial information is mediated by cortical or membrane patterns. The remainder of this paper focuses on the latter.

Endogenous Electric Fields

One way membranes can provide spatial information is by generating electric fields. Indeed, all living cells produce electric fields by transporting ions across their membranes. The sodium-potassium pump utilizes energy from ATP to move three sodium ions out of the cell while taking in only two potassium ions [23]. With each cycle of the pump the interior of the cell thus acquires a net negative charge equivalent to one electron. So the inside of every living cell is electrically negative with respect to its external environment, and the voltage across the membrane — the "membrane potential" — ranges from about 50 to 200 mV DC (average ~70 mV). This produces a steady endogenous electric field in the 10–100 mV/mm range [24].

Multicellular organisms, and their organs, are covered by an epithelium — a single layer of cells laterally connected by tight junctions that block the flow of

ions. Epithelia are polarized, in the sense that the ion channels on the side facing away from the organ or organism are different from the ion channels on the side facing the organ or organism. The result is a "transepithelial potential" that (unlike the transmembrane potential of individual cells) is usually negative on the outside of the organ or organism and positive on the inside. The transepithelial potential typically ranges from 15 to 60 mV [24].

Xenopus laevis embryos generate endogenous electric fields from the single cell stage through at least the neurula stage [25–27]. In the embryos of chicks (*Gallus gallus*) and mice (*Mus musculus*), large ionic currents pass through the primitive streak, a furrow through which cells move into the interior as they differentiate into tissues and organs [28,29].

In 1995, Riyi Shi and Richard Borgens proposed that endogenous electric fields could "both polarize the early vertebrate embryo and serve as cues for morphogenesis and pattern." If this were true, they wrote, "at least five corollaries must be satisfied: (1) embryonic cells must be responsive to extracellular voltages within the range of magnitudes measured within embryos, (2) disturbance of these endogenous gradients of voltage by imposed voltages in the physiological range should result in developmental arrest or abnormality, (3) this disturbance should be most profound at the embryonic stages when endogenous fields are present within the embryo, (4) since the internal voltages are spatially polarized during development, the form of teratological change in the embryo produced by an artificially imposed field should be predictable based on its orientation relative to the embryo's orientation, and (5) any technique that will reduce or eliminate an endogenous voltage gradient should lead to developmental arrest or retardation. All five of these requirements have been met" [30].

For example, applied electric fields of physiological strength can induce and guide cell migration *in vitro* [31–39]. Furthermore, targeted disruption of endogenous electric fields disrupts normal development in ways that suggest the fields are controlling morphogenesis [40–43]. There is also evidence that direct currents in the physiological range can affect gene expression [44,45].

(Note that this has nothing to with the controversy surrounding the alleged effects of environmental electromagnetic fields — whether extremely low frequency or microwave frequency. The endogenous electric fields that concern us here are steady, not oscillating.)

Since the topology of an endogenous electric field would depend on the spatial arrangement of ion channels in the membrane or epithelium, such a field could be one way that membrane patterns provide spatial coordinates for embryo development. Another way that membrane patterns could affect development is through intracellular signaling.

Membrane Proteins and Intracellular Signaling

Networks of intracellular signaling molecules regulate a cell's morphology, physiology. They also interface with GRNs to regulate gene expression, and they mediate a cell's response to extracellular signals such as hormones and growth factors.

Membrane proteins are key nodes in such networks. Many intracellular signals originate with them, and their spatial localization is often essential to their proper functioning. Some of the more important membrane-bound signaling molecules are the Ras proteins (so called because they were originally found in cells transformed by Rat sarcoma viruses) [46].

Ras proteins are localized mostly on the inner face of the plasma membrane, though they also occur in inner membranes such as the Golgi apparatus [47]. They come in many forms: in humans alone, the Ras superfamily includes more than 150 different members [48]. Distinct Ras isoforms have distinct functions [49], including the regulation of ion channels [50], cell migration [51], and cytoskeletal remodeling [52]. Proper Ras functioning is essential to mammalian development, and its disruption has been linked to cancer [53].

Ras proteins are organized in the membrane into spatially segregated "nanoclusters," each containing several proteins [54–56]. The spatial localization of Ras proteins in nanoclusters is essential for generating and regulating spatially distinct intracellular signaling circuits [57,58]. In 2008, Angus Harding and John Hancock wrote that those circuits "integrate and process signals to operate as switches, oscillators, logic gates, memory modules and many other types of control system. These complex processing capabilities enable cells to respond appropriately to the myriad of external cues that direct growth and development." Harding and Hancock identified "common design principles that highlight how the spatial organization of signal transduction circuits can be used as a fundamental control mechanism to modulate system outputs *in vivo*" [59].

For example, Ras nanoclusters operate as analog-digital-analog converters. Ras is either non-activated (off) or activated (on); it responds to an external signaling molecule such as epidermal growth factor by switching on; the concentration of the external signaling molecule determines how many Ras molecules are activated; and the number of activated Ras molecules determines the downstream concentration of an intracellular molecule that interacts with other signaling networks and regulates gene expression. The spatial organization of Ras molecules in nanoclusters is essential to reduce noise and produce high fidelity signal transmission across the membrane [60–62].

So spatial organization is essential to the proper functioning of membrane proteins, and those proteins can generate intracellular signals that regulate gene

expression. The gene regulatory networks described by Davidson and Carroll are related to DNA information at one end and spatial information at the other. Neither source of information can be discounted.

The Sugar Code

Cell–cell interactions — including those in developing embryos — depend on carbohydrates localized on the surface of each cell. Sugars can be attached either to lipids (glycolipids) or to membrane proteins (glycoproteins). Carbohydrate-binding proteins (lectins) mediate their interactions. Because sugars can be covalently linked in a variety of ways (unlike amino acids in a protein, which are all linked by identical peptide bonds), the diversity of side chains on glycolipids and glycoproteins is enormous.

In 1985 Ronald Schnaar wrote, "There appears to be a code on the surface of each cell that specifies its function and directs its interactions with other cells, a code in some ways comparable to the genetic code carried on the DNA molecules *inside* each cell." The "letters" of the cell surface code to which Schnaar was referring are sugar molecules. A few monosaccharide building blocks can produce the enormous diversity of "words" needed to identify the many different kinds of cells in a complex organism, Schnaar explained, because "each building block can assume several different positions. It is as if an *A* could serve as four different letters, depending on whether it was standing upright, turned upside down, or laid on either of its sides. In fact, seven simple sugars can be rearranged to form hundreds of thousands of unique words, most of which have no more than five letters. (This alphabet is even more efficient than the genetic code: the four nucleic acids that constitute DNA — guanine, adenine, thymine, and cytosine — can be connected only front to back, like roller coaster cars.) So, not only are sugars in the right place to serve as the alphabet for the cell-surface code, they have the requisite structural flexibility too." Schnaar concluded, "It may be that as much control over the cell's fate, and as much of the language of life's unfolding, reside on the cell's surface as in its nucleus" [63].

Hans-Joachim Gabius has called this the "sugar code." According to Gabius, sugars provide a "high-density coding system" that is "essential to allow cells to communicate efficiently and swiftly through complex surface interactions." This is because "all the structural requirements for forming a wide array of signals with a system of minimal size are met by oligomers of carbohydrates. These molecules surpass amino acids and nucleotides by far in information-storing capacity and serve as ligands in biorecognition processes for the transfer of information" [64,65]. In 2009, Lopez and Schnaar provided evidence that membrane patterns in

cells of the immune system and the nervous system depend in part on lateral interactions among their constituent glycolipids [66].

So the sugar code carries essential biological information in addition to that carried by DNA sequences. It is not known whether the sugar code can be directly inherited, but there is evidence that other cell surface patterns are heritable independently of DNA sequences.

Some Membrane Patterns Can Be Inherited apart from the DNA

In single-celled protozoa, changes in cilia patterns in the cortex can be inherited apart from changes in the DNA. In 1965, Beisson and Sonneborn induced one member of a conjugating pair of *Paramecium aurelia* to transfer to its partner a section of cortex that had been surgically inverted 180º relative to the surrounding cortex. The DNA was unchanged. Ciliates with artificially inverted rows have been stably maintained for thousands of generations [67,68].

In 1977, Ng and Frankel reported similar results with *Tetrahymena pyriformis* and concluded, "The cell as an architect thus not only makes use of the genomic information to produce the appropriate building blocks, but, in addition, also arranges the building blocks according to the blueprint as defined in the preexisting architecture" [69]. Frankel called this extra-genic blueprint the "corticotype" [70]. Similar results have been reported in *Tetrahymena* by Nanney and in *Stylonychia* by Grimes [71,72]. Clearly, cortical patterns in ciliates can serve as their own templates when they replicate.

There is also evidence that some cellular patterns in multicellular organisms are heritable apart from the DNA. In 1977, Albrecht-Buehler reported that after mitoses in cultured 3T3 mouse fibroblast cells, about 40% of daughter cells contained mirror symmetrical actin-bundle patterns and performed directional changes in a mirror symmetrical way. He concluded that the "organizations of daughter 3T3 cells form mirror images of each other" at the time of mitosis [73].

In 1979, Solomon observed that about 60% of sister pairs in cultured neuroblastoma cells displayed analogous morphologies. He concluded that "determinants of biologically functional shape can be dictated to some extent by the cells themselves. Such a program of information can be heritable through mitosis," though "we do not know, of course, how or in what structures this information is stored" [74]. In 1981, Solomon found additional circumstantial evidence for endogenous determinants of morphology, and he concluded, "It is possible that detailed cell morphology is specified by structures which nucleate the assembly of the

cytoskeletal fibers that underlie that morphology," though "an alternative model is that the endogenous determinants of neuroblastoma morphology may reside at the cell surface" [75].

In 1990, Locke reported paired patterns in caterpillar epidermis cells that "imply that a part of the epigenetic sequence leading to the formation of the pattern has replicated [and been] inherited by daughter cells. It is not just genetic material that is inherited but part of a cell in a particular state. Inheritance is somatic, in the sense that it is part of the operation of an epigenetic determinant that has been inherited." According to Locke, the problem with such inheritance is that it "requires more than number and kind of molecule. The duplication of pattern involves relative position and orientation," factors that "cannot be specified only by a base sequence." Locke concluded, "The observations suggest that while the detailed arrangement of cell components may be variable and not under direct genetic control, some patterns result from epigenetic determinants that replicate and are inherited from one mitosis to the next" [76]. The following year, Locke and his colleagues published "further evidence for the operation of transiently heritable factors as determinants for cell pattern" [77], and in 2007 an international team of biologists reported that similar mirror-symmetric divisions are essential for proper neural tube development in zebrafish embryos [78].

As Solomon pointed out, such symmetrical divisions may be due to the inheritance of cytoskeletal patterns, or membrane patterns, or both. In the case of membrane patterns, proteins from the cell interior are incorporated during membrane growth only if they match the existing matrix. George Palade wrote in 1983 that membranes "recognize and incorporate like components, grow by expansion in two dimensions, and eventually divide into two sets of descendant membranes, one for each daughter cell. These sets are qualitatively identical" [79].

Robert Poyton has proposed a detailed hypothesis to explain how this process might work. According to Poyton, the units of epigenetic spatial memory in membranes are hetero-oligomeric membrane proteins, of which there are many kinds. These proteins are localized on membrane surfaces in quasistable "unit areas." When phospholipids are incorporated into the membrane in preparation for replication, the hetero-oligomers dissociate into their subunits. Then newly synthesized subunits in the cytoplasm associate with the corresponding older subunits to form hybrid hetero-oligomers that are chemically identical to the originals. Thus membrane replication — like DNA replication — is semi-conservative. Poyton wrote, "It is the preexisting spatial memory encoded in a membrane that brings new proteins to its surface… Realizing that genetic memory is one-dimensional, along a DNA molecule, whereas spatial memory is likely to be two-dimensional, along membrane surfaces, and three-dimensional within the cellular interior, it is probable that spatial memory is more complicated and diverse than genetic memory" [80].

Some recently published work is consistent with several aspects of Poyton's hypothesis. First, empirical and theoretical studies indicate that the interaction of membrane proteins — in particular, the stability of homo- and hetero-dimers — is affected by the extent of their dilution in lipid bilayers [81,82]. Second, as prions demonstrate, proteins can serve as templates for their own self-replication [83–85]. Third, experiments show that membrane proteins selectively recruit other proteins to Ras nanoclusters and adjust their orientation to maintain intracellular signaling [86–89].

Implications for Modern Evolutionary Theory

Clearly, the biological information needed for embryogenesis exceeds the information encoded in DNA sequences. RNAs and proteins encoded by DNA form gene regulatory networks that are essential for development, but those networks must be localized in spatial domains for the embryo to differentiate into various cell types and organs, and those domains must be spatially ordered with respect to each other for the organism to develop its proper morphology.

Two features of cells and embryos that provide spatial cues are the membrane and the cytoskeleton. Both are composed of subunits that are encoded in DNA, but their two- or three-dimensional patterns are not determined by those subunits, just as the structure of a house is not determined by its bricks.

The arrangement of proteins and carbohydrates in a membrane is analogous to a two-dimensional code that specifies many aspects of a cell's morphology and physiology, as well as its interactions with other cells. Indeed, several membrane codes can be distinguished: the pattern of ion channels in the epithelium of an embryo generates an endogenous electric field that provides a three-dimensional coordinate system to guide migrating cells; the pattern of membrane-bound proteins such as those in the Ras family spatially organizes intracellular signaling and mediates responses to extracellular signals; and the complex pattern of carbohydrates on a cell surface is essential for cell–cell interactions.

Membrane patterns in ciliates are known to be heritable independently of the information in DNA sequences, and there is evidence that some cytoskeletal and membrane patterns in the cells of multicellular organisms can also be inherited apart from the DNA. Taken together, the data suggest that embryo development is not controlled by DNA alone, and thus that DNA mutations are not sufficient to provide raw materials for evolution.

In 1983, John Maynard Smith defended the gene-centered view of development and evolution and asserted that the DNA-independent inheritance of cortical

patterns in ciliates constituted "the only significant experimental threat" to that view [90]. It now appears that ciliates are not the only example of non-genic developmental information and DNA-independent inheritance.

One could speculate that accidental changes in membrane patterns — analogous to accidental mutations in DNA — could provide the missing raw materials for evolution. Yet two- and three-dimensional information-carrying patterns are likely to entail more specified complexity than the one-dimensional information in DNA sequences, making beneficial "mutations" in such patterns much less probable than beneficial mutations in DNA. At the very least, calculations of the time required for evolution will now have to take into account these higher dimensions of biological information.

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