

cellular or symbiotic functions, cannot be lost without harmful or fatal results. But these genes can be replaced, and perhaps ever more easily as the interaction networks of the endosymbiont reduce in complexity, thus reducing pressures on proteins to co-evolve. In a sense, when genes are transferred from symbiont or organelle to the host nuclear genome and their proteins targeted back, this is a compensatory change [4,9,14,17]. But other compensatory transfers that affect a symbiont or organelle can also involve genes transferred from unrelated donors [8,9,18]. In the case of organelles the identity of potential donors of transferred genes is not always clear, but in insects gene donors often appear to be pathogens, specifically reproductive manipulators [7–9]. These bacteria skew the number and sex ratio of offspring in infected populations in different ways, and often reside in insect germ line cells. The frequency of transfer from these groups is therefore probably due to simple chance: their cell biology includes infection of the germ line, which provides ample opportunity for gene transfer that can be passed to future generations. The same is true of single-celled eukaryotes (protists), where all newly acquired genes are taken into the germline automatically [17].

As the prevalence of intimate and stable endosymbiotic associations has become more clear, the degree to which host and endosymbiont are integrated has been revealed to be far less discontinuous than previously believed. Accordingly, the characteristics separating ‘symbiont’ from ‘organelle’ have become less clear [3,4,19,20]. There is an understandable desire to draw a distinct line between the two for simplicity, but first we must ask, does this line exist? If so, it is best drawn by evolutionary and mechanistic distinctions, not by perceived differences born of tradition, definitions, or historical contingency. Organelles were discovered first, have been studied for decades, and their bacterial origins dominated the discussion about endosymbiosis and evolution for many years. They enjoy a status apart from other biological entities: derived from bacteria, but so different as to be given their own name. But the list of their ‘unique’ characteristics is shrinking: stable endosymbioses promote extensive

genome reduction in the symbiont, HGT from various sources to the host genome to maintain symbiont function, and now the targeting of protein products from host to symbiont has even been found [4,10]. These make clean separation of endosymbiont from organelle more difficult to see, prompting us not to look for the point when a symbiont ‘becomes’ an organelle, but rather to ask, ‘Is there really anything so special about organelles?’

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Animal Evolution: Looking for the First Nervous System

The human brain is easily the most baffling bit of biology on the planet. How did the nervous system evolve? What came first: neurons or synaptic proteins? A new paper studying the pancake-shaped *Trichoplax* suggests it was not the neurons.

Erik M. Jorgensen

Something bad must have happened around 542 million years ago: the Ediacaran period, which had seen the

rise of complex, unfamiliar looking multicellular marine life forms, ended with an extinction that wiped out most of these creatures. Unfortunately, this event obscured our view on the

evolution of the first nervous systems. We know that some Ediacaran animals must have had nervous systems, as they left tracks and tunnels, and thus could move in an active, organized fashion. But because they left no directly recognizable successors, we can't infer how those first nervous systems arose. The Cambrian explosion that followed gave rise to the familiar animal phyla. Examination of their genomes suggests that the nervous system arose once, in a perfect state, as all of the key molecular components of the nervous system are conserved in the genomes of animals: the ion channels used by excitable cells, an extensive repertoire of neurotransmitters and synaptic machinery. In fact at the level of neurotransmitter systems, the human nervous system looks like a simplified version of the primordial nervous system. How then did the basic unit of the nervous system — the neuron — arise? What came first? Synaptic proteins, or the specialized anatomy of neurons? What was the first neurotransmitter? What kind of behavior did it mediate? In the last few years, evidence has arisen that some of the more primitive soft-bodied creatures around today, including the odd little *Trichoplax*, might offer a glimpse of Pre-Cambrian life. A new study by Smith and colleagues [1] in this issue of *Current Biology* describes the anatomy of *Trichoplax* in unprecedented detail and demonstrates a complicated repertoire of cells, but they are unfamiliar and bizarre.

Pre-Cambrian Animals

In the last few years the genomes of some of the more insignificant creatures on the planet have been completed — porifera, ctenophores, and placozoans. These animals are all soft-bodied and presumably sit at the base of the animal tree of life, though their exact relationship with the rest of animals is still contentious. The creatures in these phyla are quite simple, they have soft bodies, they lack three germ layers, and lack a true gut. But they can coordinate cells to move or contract. These phyla may be representatives of Precambrian animal life and could provide insight into the ancient origins of the nervous system. The sole representative of the Placozoans, *Trichoplax*, is a mere

speck of an animal. It was originally discovered in 1883 crawling on the glass of a fish tank containing seawater. Its simple circular body plan, only 1 mm in diameter, was described at the time but the organism was largely forgotten thereafter until its rediscovery in the 1970s.

Can this dog do tricks? Sure, the essential ones: the pancake that is *Trichoplax* can glide along a surface, which implies coordination of beating cilia. Moreover, when it comes across a patch of algae, it pauses over the food source, presumably digesting them by secreting enzymes onto its ventral surface, for absorption via microvilli. *Trichoplax* also changes shape from a circular to an elongated disk sometimes. So is there evidence of a nervous system underlying these behaviors? Now, through the work of Smith *et al.* [1], a full understanding of the anatomy of the ~50,000 cells in *Trichoplax* has become available. *Trichoplax* resembles a sandwich composed of two epithelial layers and some loose cells in between; there is no gut, no muscles or neurons—instead there are only six specialized cell types. It moves by means of cilia covering the ventral surface. Combined with previous genome sequence data, this study suggests that the genes for a nervous system are present, but neurons are not.

Pre-Cambrian Proteins

What are the important genes for understanding nervous system evolution? Recently the genomes of representative species from the phyla of porifera [2,3], ctenophora [4,5], and placozoa (*Trichoplax*) [6] have been sequenced. All three phyla encode the components for fast synaptic transmission: voltage-gated ion channels and specialized SNARE proteins that mediate synaptic vesicle fusion. Importantly, these genomes also encode complexin, a SNARE-binding protein, which may interrupt constitutive fusion of vesicles [7], and introduce a regulatory step. But the key protein for synaptic function is synaptotagmin, the calcium binding protein that drives regulated vesicle fusion. The homolog of synaptotagmin in ctenophores and sponges is not closely related to the synaptic version. Thus, it is not clear if calcium-dependent vesicle fusion can take place in sponges and ctenophores. By contrast, *Trichoplax*

has a protein closely related to the synaptic synaptotagmin and thus perhaps only *Trichoplax* will exhibit true synaptic behavior, i.e. calcium-activated vesicle fusion.

Surprisingly, only ctenophores possess panexin gap-junction proteins, which allow rapid communication between cells by direct electrical conductance. All three phyla, placozoa, porifera and ctenophora, lack pentameric ligand-gated ion channels altogether, and therefore show no evidence of fast neurotransmission by acetylcholine, serotonin, dopamine, GABA or glycine. It is possible that variants will eventually be found when these draft genome sequences are fully assembled. But the lack of most classical neurotransmitter receptors is further supported by a lack of the vesicular transporters for acetylcholine, monoamines, GABA, glycine, or nucleotides. These are the proteins that make a sophisticated nervous system, that provide excitatory or inhibitory inputs, and diversity of neurotransmitter responses in complicated networks — these primitive animals are deprived of access to this toolkit.

What was the first fast neurotransmitter? Probably glutamate, based on the absence of any other candidates. The genomes of all three phyla encode subunits of the glutamate receptor family. In fact, transmission using these receptors may be quite elaborate in these organisms, as the ctenophore *Pleurobrachia bachei* encodes 14 different ionotropic glutamate receptors [5]. However, it remains to be seen whether these receptors form glutamate-gated ion channels, and there is reason to doubt. Only *Trichoplax* has a protein weakly similar to the vesicular glutamate transporter; sponges and ctenophores only have homologs of the related sialic acid transporter sialin. Thus, the molecule activating these receptors may not be glutamate at all, and given the absence of a synaptotagmin-1 ortholog it is not even clear that transmission in sponges and ctenophores will be fast.

Pre-Cambrian Cells

Based on the genomic data, it appears that the most primitive organism with all of the true components for synaptic transmission is the pancake-shaped *Trichoplax*. So does *Trichoplax*

possess the cellular components of a recognizable nervous system? In the light of the new work by Smith *et al.* [1], the answer is a resounding 'No'.

Grossly, *Trichoplax* is composed of two epithelial layers, it has no endoderm, glands, musculature or recognizable nervous system. Smith *et al.* used cutting-edge methods to preserve cells and searched for evidence of neurons and synapses. Specifically, they used high-pressure freezing to preserve tissues, thus avoiding the substantial artifacts caused by slow aldehyde fixation and dehydration, and the view is stunning: there is nothing resembling a typical nervous system; there are no axons, there are no synapses. Nevertheless, the authors find evidence for specialized cells in these simple creatures: six different cell types can be identified. On the ventral surface are three cell types: the ventral epithelial, lipophil and gland cells. The cells on the lower surface are ciliated and provide for gliding motility. These cells are also covered with a shag carpet of microvilli, which serves as an external stomach to digest algae growing on the substrate. The dorsal surface is covered by a thin epithelium. Between the ventral and dorsal surfaces are the rare crystal cells and a loose collection of fiber cells.

The most bizarre cells are these crystal cells. They are found at the margin of the animal (Figure 1) between the epithelial cell layers. Each contains a specialized organelle containing a crystal that can polarize light. Are they eyes? There are reports that *Trichoplax* responds to light, and opsins are encoded in the genome [8]. But the crystal cells do not look like neurons by ultrastructure (or anything seen before, for that matter) and do not stain for antibodies for SNARE proteins — which would be required for secretory communication with other cells.

The fiber cells are also intriguing. Since they have long processes reaching among the other cells, one could imagine them to be the predecessors of neurons or muscle cells. Indeed, they express P2X purine-activated ion channels and might therefore receive excitatory input. However, the contacts with other cells lack evidence of synaptic specializations, and the processes lack a contractile apparatus, despite the presence of muscle myosins in the

genome. Moreover, these cells do not express the specialized SNARE proteins used at synapses.

Antibody staining for synaptic SNAREs instead highlighted the gland cells. The gland cells, like the ventral epithelial cells, are ciliated and therefore contribute to locomotion. Feeding animals pause over patches of algae, making it possible that these cilia are not just used for locomotion but perhaps also sensing food. Gland cells also contain potential secretory vesicles, some vesicles even appear to be docked to the plasma membrane. But the cells are oriented toward the ventral surface, making it possible that these cells secrete material onto the substrate to aid in either locomotion or digestion rather than mediating cell-cell communication.

Alternatively, of course, the gland cells may be *bona fide* neurosecretory cells releasing fast-acting neurotransmitters onto other cells. There are three potential neurotransmitters encoded in the genome of *Trichoplax*: the neuropeptide FMRFamide, the purinergic transmitter ATP, and the classical neurotransmitter glutamate. Smith and colleagues demonstrated that the gland cells express the neuropeptide FMRFamide. In most organisms, neuropeptides activate metabotropic receptors and not fast-acting ion channels. But in invertebrates FMRFamide can also activate ion channels of the ASIC family. *Trichoplax* has a particularly rich collection of genes encoding ASIC channel family members, introducing the possibility that fast neurotransmission in *Trichoplax* acts via peptide-gated ion channels rather than classical neurotransmitter-gated ion channels.

The gland cells could also use glutamate or ATP as a neurotransmitter, as the genome encodes subunits of ionotropic glutamate and P2X purinergic receptors. If glutamate or ATP is released from vesicles, then there must be a vesicular transporter. The glutamate and nucleotide transporters are encoded by the SLC17 family of anion transporters. The *Trichoplax* genome does not encode orthologs of the conserved glutamate or ATP transporters, but does encode several other SLC17 anion transporters; one of these others may load these molecules into secretory vesicles.

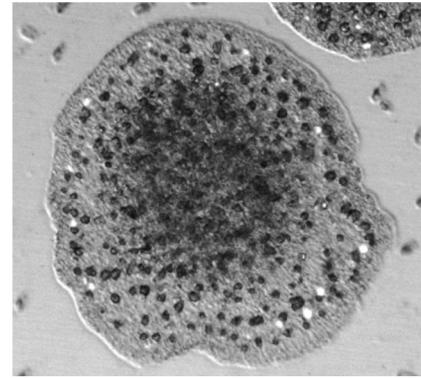


Figure 1. *Trichoplax adhaerens*. Micrograph of *Trichoplax* in partially polarized light. Crystal cells appear as white dots around edges. The dark granules in the center are inclusions in fiber cells. (Photo: Carolyn Smith.)

There is one other somewhat disturbing possibility: glutamate is not a neurotransmitter — and *Trichoplax* does not possess a primitive nervous system that uses any classical neurotransmitters. The sole evidence that glutamate is acting as a transmitter is the presence of ionotropic glutamate receptors. But these receptors may simply be relaying the presence of a nutritive signal from the algae — amino acids. Glutamate activation of the gland cells could direct movement toward a food source. In this model, amino acids evolved to become the predominant classical neurotransmitters because they were originally extrinsic molecules signaling the most important thing to the animal: the presence of something to eat. Only later did they become adapted for internal communication.

It is all a big surprise. Its genome says *Trichoplax* is an animal with a full-blown nervous system, including synaptic proteins, neurotransmitter systems, and muscle proteins; it even engages in behaviors we expect would require a neuromuscular system. But instead we find a foreign and unrecognizable cellular anatomy, no axons, no synapses, no muscles. There are three possibilities: *Trichoplax* had a nervous system but lost its anatomical specializations during the process of acquiring a simpler lifestyle. Alternatively, our expectations were wrong and a nervous system can look much different than we expected. Finally, it may have never had one, and we really are looking at what animals did with all the pieces before they were

assembled into a fabulous biological contraption.

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Tumor Models: Tumor–Stroma Interactions Drive Neoplastic Transformation in *Drosophila*

Stromal cells play a supportive role in the initiation and progression of carcinomas. A new study in *Drosophila* implicates mesenchymal cells in supporting EGF receptor-driven tumor growth and cellular transformation of epithelial tissues.

Marco Milán

Carcinomas, malignant neoplasms of epithelial origin, are the most common form of human cancer. Mesenchymal cells in the stroma regulate the expression and remodeling of the extracellular matrix (ECM) and produce growth factors that support the survival and proliferation of epithelial transformed cells. As they reported recently in *Current Biology*, Herranz et al. [1] have used the genetic model species *Drosophila* to dissect the underlying molecular and cellular mechanisms driving tumor–stroma interactions. This work underscores the contribution of resident mesenchymal cells in promoting the neoplastic transformation of EGF-receptor-expressing epithelial cells and identifies Dpp and Wingless as the signaling molecules driving growth of these two cell populations.

EGF-receptor gene amplification has been reported in a wide range of carcinomas, and mutations that activate the small G protein Ras are found in 20–25% of all human tumors. However, neither EGF-receptor overexpression nor the presence of activated Ras is sufficient to drive malignant transformation, and

additional oncogenic mutations are required for disease progression. In this regard, the imaginal primordia of *Drosophila* — monolayered epithelia within the feeding larvae that grow one-thousand fold in cell number and tissue size — have been used to identify new molecular elements that cooperate with these two oncogenes in driving tumor growth, epithelial transformation, basement membrane degradation, and invasive behavior [2–5]. Mutations that affect the Scribbled–Disc Large–Lgl cell polarity complex or those causing mitochondrial dysfunction, or overexpression of certain miRNAs, cooperate with EGF receptor/Ras dysregulation in promoting tumorigenesis. In all these cases, tumorigenesis relies on a JNK-dependent transcriptional program that regulates the invasion of transformed cells, drives the expression of the mitogenic molecules responsible for tumor growth, and induces the expression of matrix metalloproteins (MMPs) involved in basement membrane degradation, a prerequisite for tissue invasiveness [6,7].

The work of Herranz et al. [1] stems from the observation that depletion of the Polycomb group epigenetic silencer Pipsqueak, a BTB-containing

nuclear protein [8], cooperates with EGF receptor to elicit malignant neoplastic growth of imaginal primordia. The multilayered tumor induces the expression of MMP1 and the consequent degradation of the basement membrane and becomes highly metastatic, as transformed cells are found in distant internal organs such as the gut and malpighian tubules. Remarkably, Pipsqueak behaves as a tumor-promoting gene in a *Drosophila* Notch-driven epithelial tumor model [9]. This observation thus reinforces the context-dependent tumor suppressor or tumor-promoting roles of many cancer genes.

As is often the case, the initial observation made by Herranz et al. [1] that allowed the identification of a mesenchymal cell population supporting EGF-receptor-driven tumor growth was unexpected, but a key finding. During the characterization of the oncogenic cooperation between EGF receptor and Pipsqueak, GFP-positive EGF-receptor-expressing cells were found to intermingle with “groups of cells not expressing GFP” (Figure 1). Further functional characterization of this population indicated that the GFP-negative cells are resident myoblasts that proliferate in response to Dpp — a member of the TGF- β superfamily — produced by the tumor. Thus, those myoblasts abutting the transformed cell population showed strong activation of the Dpp pathway and elevated mitotic activity.

Genetically elegant experiments performed by Herranz et al. [1] demonstrated that the proliferative myoblast population plays a major role in driving neoplastic tumor growth. Thus, selective ablation of the myoblasts